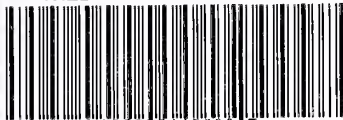


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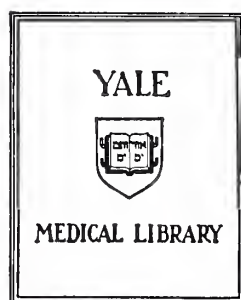
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INVESTIGATION OF PREDICTIVE FACTORS IN KELOID FORMATION

Cherise Malinda Dyal

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1989





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INVESTIGATION OF PREDICTIVE FACTORS IN KELOID FORMATION

A Thesis Submitted to the Yale University
School of Medicine in Partial Fulfillment
of the Requirements for the Degree of
Doctor of Medicine

by

Cherise Malinda Dyal

1989

ABSTRACT

INVESTIGATION OF PREDICTIVE FACTORS IN KELOID FORMATION. Cherise M. Dyal.
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The literature on factors associated with keloid formation has thus far been conflicting. This study was undertaken to investigate the possible association of HLA Class I (A, B, C) histocompatibility antigens; ABO/Rh blood groups; and patient and family medical history with the development of earlobe keloids. These factors were investigated in two groups of Black females. The keloid former group consisted of 20 patients with unilateral or bilateral earlobe keloids (the only indisputable form of keloids). The control group consisted of 20 women who had undergone earpiercing and were free of any objectionable scars. Blood samples obtained from both groups were typed for HLA haplotype and ABO/Rh blood group. All other factors were assessed by questionnaire.

Factors found to occur in greater frequency in the keloid-former group and indicating increased risk for keloid formation included: possession of the HLA-A 9 (relative risk = 4.636), HLA-A 23 (9) (relative risk = 4.846), HLA-Aw 34 (relative risk = 8.200), or HLA-Cw 2 (relative risk = 6.333) antigens; history of hypertension (relative risk = 9.400) and history of infection immediately after ear piercing (relative risk = 6.667). Factors found in greater frequency in the control group and suggestive of a protective effect against the formation of keloids included: possession of the HLA-A 2 antigen (relative risk = 0.206); a positive history of non-drug allergies (relative risk = 0.327); and family history of tuberculosis (relative risk = 0.143). Differences were also found between the two groups in the age at time of ear piercing with the control group being younger (median age 4 vs. 17 years; mean age 8.54 vs. 20.58 years).

These results suggest that there may be factors which can be considered to increase risk for the formation of keloids while there others maybe protective against

keloid development. Further research into the predictive factors in keloid formation should be undertaken and the factors indicated in this study might serve as a basis for these future investigative efforts.

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INTRODUCTION

NORMAL WOUND HEALING

A wound is defined as the disruption of the physical integrity of any of the tissues of the body, especially that caused by physical means and with interruption of continuity (Stedman, 1982). Wound healing is the process by which the tissues of the body repair the damage caused by the trauma and re-establish continuity which has been interrupted. Healing is accomplished through a series of complex biological processes, the nature of which is determined by the status of the wound edges after injury. A wound is said to heal by first intention if the wound edges are approximated after wounding, either immediately or some point later. Wound repair which is performed days after wounding is known as delayed primary closure. A wound heals by second intention if the wound receives no surgical intervention and natural biologic processes are allowed to restore continuity of wound edges (Carrico et al., 1984).

In general, there are two mechanisms through which continuity is re-established. If the tissues involved are confined to the epidermis then the process is one of regeneration known as epithelialization or epidermal healing which involves proliferation and migration of epithelial cells across the wound which originate from underlying epithelial cells. This process is thought to be stimulated by loss of contact inhibition which normally keeps the intact epithelium in a state of mitotic balance. Factors responsible for proliferation and differentiation as well as inhibition of the the epithelium have been demonstrated. Human epidermal growth factor (hEGF), a polypeptide which is up regulatory in nature, has been isolated from human urine (Starkey et al., 1975). This substance functions as a mitogen through receptors on epidermal cell membrane and stimulates differentiation and initiates DNA and RNA synthesis. Glycosaminoglycan (GAG) production by fibroblasts through fibroblast

receptors is also stimulated. More rapid de-epithelialization and wound maturation have been shown to occur when hEGF is applied to a wound (Franklin & Lynch, 1979).

The existence of down regulatory substances which function as endogenous inhibitors of mitotic activity in normal intact epidermis have also been demonstrated and have been given the name chalones, from the Greek "chaleo" meaning "to lower or reduce speed" (Bullough & Lawrence, 1960). These substances are glycoproteins which are phase specific in that they inhibit the G1 or G2 phases of mitotic cycle. Their actions are reversible and removal of the chalones terminates their action.

Mesodermally derived tissues deep to the epidermis do not have the capacity to regenerate. These tissues heal through scar formation, a process known as mesenchymal healing. The dynamics of scar formation can be divided into four phases. The first is the hemostatic phase and is characterized by platelet aggregation and clot formation. The platelets, activated by thrombin, release platelet derived growth factor which stimulates angiogenesis and collagen synthesis by fibroblasts (Cerra, 1982).

The second phase is inflammatory, and is characterized by infection control and microscopic debridement through phagocytosis (Abramson et al., 1984). Many cells are involved in this phase of wound healing (Boucek, 1984). Polymorphonuclear cells (PMNs) or neutrophilic leukocytes immigrate into the tissue spaces attracted by complement which acts as its chemotactic factor and while the absence of PMNs does not affect primary wound healing it does increase the risk of wound infection. Lymphocytes are also attracted to the wound under the chemotactic influence of complement and in addition to their role in infection control they serve to provide factors which act as local messengers. These substances include lymphokines which may suppress hypersensitivity reactions, macrophage migration inhibition factor, and other enzymes whose products serve as mitogenic chemical messengers in the repair process (Boucek,

1984). Macrophages are believed to be the primary source of one or more signals in the repair process which stimulate fibroblast proliferation and angiogenesis (Hunt, 1985) as well as elaborate the neutral proteases collagenase and elastase which function in collagen phagocytosis (Boucek, 1984). Macrophages are the predominant cells in the inflammatory phase and as facultative anaerobes they elaborate lactic acid which is a stimulant of collagen synthesis. Elimination of macrophages will inhibit wound healing (Hunt, 1982). Mast cells which contain histamine as well as eosinophils are also involved in this phase of wound healing and the eosinophil-mast cell-PMN interaction may involve phospholipase D which inactivates the platelet-activating factor of mast cells (Boucek, 1984).

The third phase of mesenchymal healing is the proliferative phase and is characterized by deposition of ground substance followed by collagen deposition (Pospisilova, 1982). Glycosaminoglycans (GAGs) which are acidic polysaccharides are the primary constituents of ground substance and may modulate collagen production and organization. Fibroblasts which are stimulated to proliferate by macrophages are responsible for the production of procollagen which is later converted to tropocollagen and modified to its final state of collagen which is the substance of which mature scar is made.

The fourth and final phase of scar formation is that of remodeling which is characterized by collagen lysis and collagen resynthesis. This phase occurs over the course of many months and is responsible for achieving the ultimate tensile strength of the scar tissue through collagen degradation of the initially randomly oriented collagen fibrils which are more susceptible to lysis and the production of new collagen into bundles which are oriented with the lines of stress. This orientation of collagen fibrils confers greater tensile strength to the scar and makes the collagen in the scar less susceptible to degradation.

FACTORS AFFECTING WOUND HEALING

Intrinsic factors which modulate wound healing are many. There are at least six classes of endogenous substances which have been shown to have some effect on the process of wound healing (Boucek, 1984). Histamine has been shown to enhance the permeability of arterioles, capillaries and venules to albumin, globulin and fibrinogen by inducing the contraction of endothelial cells. Through the increase in permeability, histamine plays a role in modulating the hemostatic and inflammatory phases of scar production. While the precise role of serotonin in healing has been controversial it has been shown to be more effective than histamine in increasing capillary permeability in some species and may also cause contraction of arterial and venous smooth muscle and arteriolar dilation thereby having an effect on the hemostatic and inflammatory phases of healing (Boucek, 1984). Mild tissue injury has been shown to cause the release of norepinephrine into the arterial wall which through adrenergic receptors cause irregularities in the endothelial folds and the appearance of edematous clear vacuoles in the endothelial cells which enhance platelet and leukocyte adherence to the vascular surface (Boucek, 1984). Platelet aggregation results in activation of the membrane enzyme phospholipase A₂ which causes the release of arachidonic acid into the extracellular spaces leading to the ultimate formation of several prostaglandins which mediate cellular migration during the initial steps of inflammation. Bradykinin has been long known to be a potent local tissue hormone which mediates Celsus' four cardinal signs of inflammation: *rubor et tumor cum calore et dolore*, "redness and swelling with heat and pain". Finally, superoxide dismutases which are needed to convert the toxic products of respiring cells are essential to wound healing since without them superoxide radicals adversely affect the activities of fibroblasts and macrophages in the repair process (Boucek, 1984).

Wound healing is influenced by disease states. The immunocompetence of the organism has an indirect effect on wound healing. While leukopenia at the time of wounding and the inflammatory phase has no effect on subsequent cellular wound debridement, fibroblast proliferation, or connective tissue formation the absence of PMNs during the inflammatory phase can predispose to wound infection (Carrico et al., 1984). Wound infection prolongs the inflammatory phase and retards wound healing although the extent to which the delay is caused by the very presence of bacteria in the wound or by host response to bacteria is still unclear (Reed & Clark, 1985). Coagulation causes platelet aggregation (an important goal of the hemostatic phase of healing) and the release mediators that increase vascular permeability and activate cells for new tissue formation. Therefore, coagulation disorders including hemophilia causing poorly formed fibrin networks and recurrent hemorrhage, Von Willebrand's disease resulting in abnormal platelet adhesion, and deficiencies of factors IX, XI, and XIII as well as fibrinogen prolonging bleeding times and delaying fibrin matrix formation can all adversely affect wound healing (Reed & Clark, 1985). Similarly liver diseases resulting in the reduction of factors involved in coagulation cause delayed wound healing. Diabetes has been shown to result in wound hypoxia both by microangiopathy and major arterial occlusion secondary to accelerated arteriosclerosis (Carrico et al., 1984). In addition, insulin has been demonstrated to be important in the early inflammatory phases of wound healing and is felt to be associated with defective leukocyte function (Carrico et al., 1984).

Collagen synthesis is the root of proper wound healing and factors which impair this will adversely affect healing. Oxygen is essential for the formation of hydroxyprolyl and hydroxylysyl residues needed for collagen. Anoxia and hypoxia will not only deprive the wound of the oxygen necessary for collagen synthesis but also decrease cell migration and proliferation, angiogenesis, and bacterial resistance (Reed &

Clark, 1985; Carrico et al., 1984). Similarly, a decrease in circulating blood volume will decrease the oxygen tension as well as the inflammatory response and delay wound healing (Carrico et al., 1984).

Aging affects all phases of wound healing causing decreased inflammation, wound contraction, cell proliferation and cell metabolism. Reduced rates of capillary ingrowth, and mast cell numbers also result. Synthesis and degradation of collagen decreases, cross-links within the collagen molecule become more stable and the collagen molecule itself becomes more resistant to degradation. In addition re-epithelialization is slowed (Reed & Clark, 1985).

Finally, intrinsic factors related to the wound itself affect healing. Tension in the wound accentuates the proliferative phase and collagen deposition (Reed & Clark, 1985). Desiccation has also been shown to delay epidermal cell proliferation and migration (Reed & Clark, 1985).

Extrinsic factors have also been shown to affect the rate of wound healing as well as the quality of the scar produced. Several nutritional deficiencies have been implicated. Severe malnutrition has been found to result in profound reduction in the strength of abdominal and skin wounds (Carrico et al, 1984). Deficiencies in a number of vitamins have proved important (Reed & Clark, 1985). Vitamin A deficiency results in delayed reepithelialization and collagen synthesis, decreased collagen stability and increased frequency of bacterial, viral, and protozoan infections. Deficiency in Vitamin C leads to the formation of unstable collagen which is subject to intracellular and extracellular degradation. In addition, macrophage migration, superoxide formation, neutrophil function and synthesis of immunoglobulin as well as complement are impaired and thus affect the inflammatory phase of healing and compromise resistance to infection. Vitamin K deficiency is associated with a bleeding diathesis and affects the hemostatic phase. A deficiency in Vitamin E increases the availability of potentially

toxic free radicals. Since Vitamin E is an antioxidant, its effects are seen in the early phases when phagocytosis generates these radicals which may inhibit fibroblast growth. Chronic zinc deficiency causes delays in wound repair and the mechanism is thought to be related to impaired synthesis of nucleic acid and protein including collagen (Boucek, 1984).

The list of drugs which can affect wound healing is long and diverse. Corticosteroids in large doses have been shown to reduce wound healing and decrease the tensile strength of wounds. This effect may be due to decreased fibroblast proliferation, reduction of granulation tissue, reduction of inflammation, reduction in oxygen and nutrient supply secondary to vasoconstriction, and by their antimitotic activity (Boucek, 1984; Reed & Clark, 1985). Drugs which inhibit protein synthesis (antimetabolites), coagulation (Coumadin), inflammation (colchicine, non-steroidal anti-inflammatory drugs, and radiation) or which limit the concentration of histamine and serotonin in wounds (hypnotics) have all been shown to adversely affect wound healing (Reed & Clark, 1985; Boucek, 1984).

Finally, the presence of a foreign body has been long known to delay wound healing. Oxygen tension in a wound is lowered to zero in the presence of a foreign body and this inhibits tissue repair, prolongs inflammation and promotes bacterial infection (Reed & Clark, 1985).

ABERRANT WOUND HEALING

Aberrant wound healing can result either from net scar underproduction or net scar overproduction. Scar underproduction can be the result of failure of any of the phases of wound healing and results in functional failure of the scar. Scar overproduction, on the other hand, leads to the development of the common conditions of hypertrophic scars (HTS) and keloids. These states are marked by deposition of collagen in excess of lysis and have been believed to be due to prolonged phases of fibroplasia and

collagen production (Oluwasanmi, 1974). Open wounds and the associated bacterial contamination can lead to states of scar overproduction.

Hypertrophic scars are raised, erythematous scars which do not extend beyond the dimension of the original injury. They are characterized by the attainment of maximal dimensions followed by spontaneous cessation of growth, the tendency toward maturation with time (Rudolph, 1987; Conway, 1960). They may regress spontaneously (Asboe-Hanson, 1960). While it has been reported that they do not tend to itch (Asboe-Hanson, 1960), patients often report otherwise.

The word keloid was first proposed by Jean Louis Alibert, a French dermatologist, who called these examples of net scar overproduction "cheloides" from the Greek meaning "claw" in describing the leg-like projections into the surrounding skin in 1806 (Goldwyn, 1981). They have been defined as "benign outgrowths having their origin in the subpapillary layer of dermis" (Koonin, 1964) and are localized to the reticular layer or corium as a poorly circumscribed mass of connective tissue composed of interlacing bundles of hyalinized collagen (Koonin, 1964).

Keloids are characterized by scar tissue which extends or overflows into normal tissues, does not regress with time, and has a tendency for recurrence post treatment (Ketchum et al., 1974). The amount of scar bears no relationship to the preceding traumatic or inflammatory. Itching and pain may be severe (Ketchum et al., 1974).

Although many investigators have failed to differentiate between HTS and keloids, those who have sought differences have often found many common features. Both Davies (1985) and Doyle and Passey (1975) found that there were no histologic differences between the two entities except for quantitative ones. Studies on collagen production have shown elevated rates of collagen production in both as indicated by measurements of proline hydroxylase activity of skin, HTS and keloid (Cohen et al., 1972). Craig (1978) found that the initial rate of collagen synthesis was 2x that of normal scar but

that the rate of synthesis decreases to approximately the same level as in normal scar 2-3 years post wounding in both HTS and keloids. Cohen et al. (1978) reported that alpha-globulins which are collagenase inhibitors were present in significant number of keloid and HTS as compared to normal skin and normal scar. Cohen et al. (1972) found increased levels of histamine in both.

Studies on the enzymatic activities of both HTS and keloids have demonstrated many similarities. Increased levels of the collagen cross-linking enzyme lysyl oxidase have been reported (Hayakawa et al., 1976; Knapp et al., 1977). Hoopes et al. (1971) have demonstrated a 2-4 x increase in the activity of glucose 6-phosphate dehydrogenase and uridinediphosphate-glucose dehydrogenase to a magnitude similar to that demonstrated in carcinogen-induced hyperplastic epithelium in studies of epithelium of HTS and keloids. These investigators also found that in dermis: (1) G6PD activity was increased 9-13x normal in HTS and keloid (this degree of increase was similar to that noted in malignant tissues of keratoacanthoma and melanoma) [since there is impairment of wound healing in G6PD deficient patients, this is of interest] (2) DNA content of 2x normal in dermis of HTS and keloids (3) glycolytic enzyme activity with the exception of phosphofructokinase was increased markedly (4) hexokinase and pyruvate kinase activities were 2-4x normal in both HTS and keloids (5) there was high glyceraldehyde 3-phosphate dehydrogenase activity in both lesions where no measurable activity was found in normal dermis (6) the 2 enzymes assayed as representative of TCA cycle (isocitrate dehydrogenase and malate dehydrogenase) demonstrated 70-150% increase in activities (7) B-hydroxybutyryl CoA dehydrogenase and alanine and aspartate transaminase activities were considerably decreased (8) increased phosphorylase activity was found. All of this was compatible with hyperplastic alterations in epithelial cells and may reflect demand to meet increased energy requirements for increased collagen formation. Of all of the enzymes

studied only alanine transaminase may be applicable to differential diagnosis between HTS and keloid since it is increased 2x normal in keloid and not at all in HTS.

It is often useful to the surgeon faced with treatment of HTS and keloids to be able to differentiate between these two entities. Researchers have dedicated many studies to the characterization of features unique to each. Five categories of unique biochemical and histologic features of HTS have been discussed in the literature. The production and arrangement of collagen in HTS has been extensively studied. While Asboe-Hansen (1960) reported that collagen fibers were arranged in bundles as in normal fibrous tissue, Knapp et al. (1977) described the collagen bundles as flatter and less clearly demarcated and reported bundle interrelationships to be less structured although the majority still ran parallel to the epithelial surface. Studies on the production of collagen have reported rates to be as high as 7x that of normal skin (Ketchum et al., 1974).

Many have studied fibroblast populations of HTS and results have been controversial. Conway et al. (1960) described three types of fibroblasts. The Type 1 fibroblast was a small, spindle shaped, highly mobile cell with a large number of mitochondria which was devoid of lipid granules. This type was found to be the largest in number. Type 2 fibroblasts which are not found in HTS were said to be much larger, less motile, and more epitheloid in shape. Normal fibroblasts (intermediate cell type) were noted to be morphologically similar to fetal fibroblasts. Knapp et al. (1977) also reported 3 cell types: the S cell (60%) which is a small, spindle shaped cell, the A Cell (35%) which is a larger, flatter, ameboid appearing cell and the K cell (5%) which is an intermediate size, unipolar cell.

Ground substance composition has been found to be unique in HTS in the increased GAG chondroitin-4-sulfate and in the decreased dermatan sulfate (Shetlar et al., 1972). Lower levels of linoleic acid were found in epidermis over HTS (Shakespeare &

Strange, 1982). On a macrostructural level occluded microvessels have been found to be a routine characteristic of HTS (Rudolph, 1987).

Unique biochemical and histologic features of keloids have also been extensively reviewed in the literature. Many studies have centered on the synthesis and orientation of collagen. Koonin (1964), Sakata (1974), and Knapp et al. (1977) described the orientation of collagen fibrils in keloids as completely random and pointed out that discrete collagen bundle formation is virtually absent. Cohen (1977) reported that keloid fibroblasts were found to synthesize collagen at an absolute and relative rate 2-3x greater than controls while fibroblasts taken from normal appearing skin adjacent to the keloid synthesized collagen in the same range as normal skin from non-keloid controls. The researchers concluded that increased collagen synthesis is secondary to autonomous capacity of keloid fibroblasts to synthesize collagen at significantly increased rates rather than increased fibroplasia. Ketchum et al. (1974) reported much higher levels of collagen production at a rate 20x that of normal skin.

Abergel et al. (1975) found that the ratio of genetically distinct collagen type I/III was significantly increased in comparison to normal human skin (type I significantly increased and type III significantly decreased). Five out of nine keloid fibroblast cultures demonstrated increased procollagen production and in these cultures there was elevated prolyl hydroxylase activity. Type I procollagen mRNA levels were significantly increased in four keloid fibroblast lines and a good correlation between mRNA levels and rate of procollagen production in the same cultures was noted which suggested regulation of collagen gene expression on the transcriptional level. These researchers, however, did point out that while not all fibroblasts may show increased collagen production this could be due to the fact that keloids are multicellular in origin (based on patients heterozygous for X-linked glucose 6-phosphate dehydrogenase (Moulton-Levy et al., 1984)) and some fibroblast cultures may represent high collagen

producing fibroblasts while others are normal collagen producing fibroblasts.

Similarly, Uitto et al. (1985) found that some but not all keloid fibroblast cultures produced increased production of type I procollagen with parallel increase in type I procollagen-specific mRNA levels. In this study type III procollagen mRNA levels remained unaltered resulting in an increased ratio of type I/type III procollagen mRNA. Ala-Kokko et al. (1987) studied regulation of collagen gene expression in keloids and fibroblast cultures and found mean procollagen production rate in keloid fibroblasts to be at control levels with slight increases in type I procollagen mRNA in keloid cell lines.

Fibroblast studies have resulted in the elucidation of some distinguishing features of keloids. Conway et al. (1960) defined 3 types of fibroblast populations as described earlier. However, unlike HTS cultures 91.2% of keloid cultures had Type 2 fibroblasts. Conway et al. (1959) found 3 fibroblast types cultured from keloid cells, two of which were not present in normal scar as described in the HTS section. Oluwasanmi (1974), however, did not find any abnormal fibroblast types. Knapp et al. (1977) found a different proportion of the types of fibroblasts found in keloids: A cell fibroblast (60%) > K cell fibroblasts (30%) > S cell fibroblasts (10%) than that which was found for HTS. Studies performed by Russel and Witt (1976), however, found no differences observed in cultured fibroblasts from keloid, normal skin and scar patients in (a) the initial period of culture initiation (b) the relative number of morphologically distinct types among different strains (c) the average cell density (d) the relative proportion of cells of different sizes between normal and keloid strains and (e) the average cell volumes.

Studies concerning the composition of keloid tissue in comparison to HTS, normal scar and skin have shown qualitative and quantitative differences, the basis of which have not been clearly defined. Cohen et al. (1972) as well as Topol et al. (1981) found elevated histamine content of keloid tissue. The work of Psillakis et al. (1971)

demonstrated significantly less potassium, magnesium and iron in keloid than normal skin; significantly more sodium, magnesium and calcium in keloid than in normal scar; and significantly less copper in keloid and normal scar than in normal skin.

Enzyme studies have also helped to demonstrate differences in keloids as compared to HTS. Increases in the enzyme proline hydroxylase used in collagen synthesis have been reported (Shakespeare & Strange, 1982). Collagenase activity has been reported to be 2x that of HTS. Finally, increased acid phosphatase activity has been found in keloids [abundant distribution of acid phosphatase has observed in keratogenous zones of epidermis and hair follicles and in center of sebaceous glands] (Im & Hoopes, 1971).

FACTORS ASSOCIATED WITH KELOID FORMATION

The literature is replete with reports detailing factors associated with keloid formation. While a few of the reports are based on studies performed, most are anecdotal in nature.

Tension in healing wounds has long been reported to be associated with aberrant scar formation. Koonin (1964) observed that with long-standing edema the mucopolysaccharide content of skin was maintained at a high level which stimulated collagen deposition. Transplantation studies involving removing keloid scars and grafting them into an area of relatively little tension showed that the subcutaneous tissue of the keloid would atrophy (Ketchum et al., 1974). Later it was reported that tension in healing wounds increased synthesis and deposition of collagen predisposing to keloids and HTS (Doyle & Passey, 1975).

Keloids have been reported to be associated with alterations in hormonal status. Noting that keloids often developed with puberty and may have peak incidence in immediate post pubertal years (Moustafa and Abdel-Fattah, 1975), that there have been

cases of regression with menopause (Koonin, 1964) and that they have appeared or increased in size during pregnancy (Ramakrishnan, 1974), researchers have suggested that keloid formation may be associated with estrogen levels in the body. It has been postulated that estrogens may alter polymerization of acid mucopolysaccharides and thereby have profound effects on physiochemical properties of ground substance (Moustafa and Abdel-Fattah, 1975). Ford et al. (1983) reported that keloid tissue in 2/3 of the female patients studied showed slight estrogen receptor activity. However, suppression of the ovaries does not alter keloid course (Koonin, 1964).

Androgens have been postulated to be associated with keloid formation. Based on the observation that keloids have predilection for chest, upper back, groin, neck and head and that these areas have demonstrated increased rate of dihydrotestosterone metabolism, Ford et al. (1983) studied androgen, estrogen and progesterone binding activities in keloid and para-keloid tissues. It was found that there was elevated androgen binding in all keloid tissue while estrogen and progesterone receptor binding was not detected in any keloid tissue from males. Other hormones such as thyroid stimulating hormone (TSH) and growth hormone (GH) have also been associated with keloid formation based on the observation that acromegalics have increased susceptibility to develop keloids which may be secondary to increased levels of TSH and GH (Koonin, 1964; Ketchum et al., 1974).

Observations have long been made on the association of keloids with pigmentation. Koonin (1964) noted that keloids were most common in Blacks with Hindus, Malaysians and other dark skinned people showing predisposition and that whites from Mediterranean areas (and consequently darker than those from less sunny areas) were more prone. Oluwasanmi (1974) and Morgan, Giolchrest and Goldwyn (1975) both reported that there have been no cases of keloids documented in albinos. Many papers have reported on the ratio of incidence of keloids in Blacks vs. whites and in their review

of keloids Doyle & Passey (1975) reported that this ratio has been found to vary from 2:1 to as great as 19:1. Morgan, Gilchrest and Goldwyn (1975) made the interesting observation that while the incidence of keloids is higher during times of physiological hyperactivity of the pituitary this hyperactivity is also associated with increased pigmentation. They went one step further to suggest that intradermal injection of triamcinolone used to treat keloids and associated with depigmentation at the local site of injection may be effective due to a local direct inhibition of melanocytes or may be caused by suppression of MSH release from pituitary by absorption of the triamcinolone. Addison's Disease (primary adrenocortical deficiency) is associated with hyperpigmentation of the skin. This has been found to result from overproduction of MSH and ACTH as a result of decreased output of cortisol by the adrenals (Harrisons, 1987). It is interesting to note that despite its association with increased pigmentation there is nothing in the literature on its association with keloids.

Many have commented on the regional distribution of keloids. Based on observations of keloid patients and the areas in which these lesions occur it has been felt that there are areas of high and low risk. Those areas which are felt to be areas of high risk for keloid development include the presternum, upper back, shoulders, neck, face, earlobes and anterior chest wall (Koonin, 1964; Crockett, 1964; Alhady and Sivanantharajah, 1969). The areas of the body which have been reported to rarely, if ever, form keloids include the eyelids, genitalia, glabrous skin of palms and soles, lower back, and lower limb (Crockett, 1964).

An inverse relationship has been noted between age and keloid and HTS development. Alhady and Sivanantharajah (1969) reported that of 182 lesions studied in West Malaysia 80% occurred in patients under the age of 30. Ketchum et al. (1974) observed that 88% of lesions in their study occurred in patients less than 30 years old, and of the 1000 cases studied by Ramakrishnan (1974) 65% occurred in patients

between the ages of 11 and 30. Doyle and Passey (1975) postulated that this relationship may be due to the inherent resiliency, and therefore, increased tension in younger skin.

Gender has been a controversial issue in its relationship to keloid formation. While some authors have written that the female to male ratio is 1:1 (Alhady and Sivantharajah, 1969), others have reported that there is increased incidence in females (Koonin, 1964; Salzman, 1970; Doyle and Passey, 1975). However, it must not be overlooked that the question may not be whether women are more prone keloids but rather just more conscious of the condition.

There have been several studies which have suggested that blood antigens may be associated with keloid formation. Studies on ABO blood type associations have been controversial. Ramakrishnan (1974) performed a study of 286 Indian keloid patients in city of Madras where most the common blood type was O and found a preponderance of keloid formers were type A. This difference was reported to be statistically significant. Ghosh et al. (1979), however, found that blood group B was preponderant in another keloid group in a study conducted in a region where group B blood was the most common. This preponderance was not considered statistically significant.

Over the past ten years questions have been raised about the association of HLA histocompatibility antigens and keloids. In 1977, Laurentaci and Dioguardi studied 40 patients with keloids and 131 controls studied all of whom were White and found that the frequency of HLA-B14 in keloid individuals was 25% as opposed to 3.8% in controls which conferred a relative risk = 6.30x. In the same study it was demonstrated that the frequency of HLA-Bw16 was 25% in keloid individuals as compared to 6.1% in controls which conferred a relative risk = 3.84x. In 1978 these same investigators studied 25 individuals with keloids and 131 normal controls and again found increased frequency of HLA-B14 (25% vs. 4%) conferring a relative risk = 6.30x and an increased frequency

of HLA-Bw16 (31% vs. 6%) conferring a relative risk = 4.85x. Based on these studies it was concluded that individuals with HLA-B14 or HLA-Bw16 were at greater risk to develop keloids. However, Cohen et al. (1979) found that there was a greater frequency in Black keloid formers of the HLA-A2 antigen (42 to 62 percent greater frequency in keloid patients conferring a 2.04x risk factor) and that there was a negative correlation between expression of HLA-Bw35 and the formation of keloids. Neither of these results was found to be statistically significant.

Attention has been focused on the area of immunology and associations with keloid formation. It was once thought that tuberculosis and syphilis were associated with keloids, but this is no longer felt to be true (Koonin, 1964). More recently studies have focused on serum and tissue levels of immunoglobulins and complement. Oluwasanmi (1974) reported the presence IgG deposits along collagen fibers. Cohen et al. (1979) studied serum and tissue IgG, IgM, and complement measurements in keloid patients and controls. They found: (1) increased levels of IgG extracted from keloid tissue suggesting a local immune process (2) significantly lower levels of serum IgM levels in keloid group but the range of values was too large to be useful for a clinical diagnostic index (3) that the C1, C3 and C4 levels of keloid patients were within normal range and (4) that IgG deposits have could be found along collagen fibers. In 1983, Kischer et al. studied serum immunoglobulin measurements, tissue immunoglobulin measurements, and immunofluorescence of IgG, IgA, and IgM in patients with keloids, HTS, normal patients and patients with normal scar. Results of this study indicated that there were increased levels of all Ig's studied (M, A, G) in keloid and hypertrophic scar tissue as compared with normal skin and that there was more IgM in keloid and hypertrophic scar tissue as determined by immunofluorescent staining. Bloch et al. (1984) studied keloid patients in comparison to non-keloid patients and found: (1) levels of IgM and C3 in serum were significantly higher in the keloid group than in the non-keloid group in which the IgA

and C4 levels were higher; (2) mitogenic response was higher in the non-keloid group to PHA (phytohemagglutinin) and Con-A (concanavalin) [T-cell mitogens].

POSSIBLE ETIOLOGIES OF KELOID FORMATION

Researchers have postulated many possible hypotheses relative to the etiology of keloid formation. While many sound attractive none have been proven. The following summarizes those hypotheses reported in the literature.

It has often been suggested that genetic predisposition may play a role in keloid formation. Ramakrishnan (1974) in his study of 1000 cases studied found that in 19 families multiple cases were observed. Koonin (1964) suggested that there may be hereditary physiochemical differences in subjects which cause overproduction of connective tissue at sites of injury this has been called fibroplastic diathesis. As of yet there has been no study which has conclusively shown an inheritance pattern associated with keloid formation.

It has been hypothesized that keloid formation may be related to an aberration of MSH metabolism (Koonin, 1964). This was based on the observations that:

(1) there is increased incidence of keloid in dark-skinned races whose melanocytes are more reactive to MSH;

(2) deeply pigmented people of all races seem more prone to keloid formation than fair skinned people;

(3) hyperpituitarism (as in acromegalia) is associated with keloid formation;

(4) the incidence of keloids is higher in states of hyperactivity of the pituitary (puberty, pregnancy) and these times are also associated with increased pigmentation;

(5) the main sites of keloid formation are in areas where melanocyte concentration is greatest (face and neck) and rare where melanocyte concentration is lowest (palms and soles of feet);

(6) there is an association of keloids to thyroid disease (hyperthyroidism) and increased pigmentation in certain cases of hyperthyroidism; and

(7) there is a negative association of keloids with corticosteroids which are inhibitors of MSH output by the pituitary in that local injections of hydrocortisones causes keloid regression and decreases in the frequency of keloid recurrence.

Other hormones have also been postulated as etiologic factors. Asboe-Hanson (1960) suggested a role for growth hormone and thyrotropin based on the observations that acromegalic patients (who have excess of GH), Blacks and children can develop spontaneous and cicatricial keloids, and that young patients thyroidectomized for Grave's disease were apt to form keloids in the operative wound. This hypothesis can be said to be supported by the fact that GH can stimulate the formation of new connective tissue, especially the formation and deposition of collagen fibrils and that thyrotropin stimulates synthesis and cellular release of mucopolysaccharides to ground substance. These effects influence tissue cells directly rather than via the thyroid.

Long standing edema has been implicated as a possible etiology based on the fact that it will delay water removal and that mucopolysaccharide content is maintained which stimulates deposition of collagen fibrils whose fibrils remain ensheathed by mucopolysaccharide (Asboe-Hanson, 1960). This researcher also observed that once deposited the breakdown of collagen is very slow but that it is uncertain as to what determines the tumor-like growth of keloids.

An interesting hypothesis has been the role of histamine as a possible cause of keloid formation. Increased numbers of mast cells containing mucopolysaccharide of the hyaluronic acid type and sulfomucopolysaccharide of the heparin type have been found at

keloidal sites as compared with normal skin and hypertrophic scar (Asboe-Hanson, 1960). Cohen et al. (1972) found evidence that histamine may be a stimulant for collagen formation and that HTS and keloids have increased histamine levels which parallels the rate of collagen synthesis. Significantly increased levels of histamine have been demonstrated in keloids and hypertrophic scar as compared with normal skin and normal scar with the greatest histamine content being found in keloids (Cohen & McCoy, 1981). Topol et al. (1981) studied response of fibroblasts from keloid tissue as compared to normal skin to histamine. They demonstrated (1) increased histamine content in keloids (2) that the growth plateau of a high percentage of fibroblast strains cultured in histamine enriched media was elevated from 50-300% over control cultures (3) that strains derived from keloid tissue were more responsive to histamine than those derived from normal skin (4) that histamine has been shown to stimulate cell growth in human fibroblasts in a dose-related fashion (60% of fibroblast studied) (5) that no difference was found in the cell response to histamine based on age or sex of patient, body location of excised tissue or duration of scar or keloid. Based on these results they proposed that keloid formers were divided into two groups, those that were histamine sensitive and those that were not. They pointed out that keloid strains which showed augmented growth plateau also showed growth suppression when pharmacologic levels of diphenhydramine was added to the medium containing histamine, but that this did not reduce growth to below control levels. It has, however, been pointed out in a criticism of this work that while 3 out of 6 of keloid fibroblasts showed stimulation by histamine 4 out of 5 of normal skin cultures also showed such stimulation and that histamine non-responding strains were not tested for growth inhibition (Cosman, 1982).

The role of sebum as a possible etiologic agent in keloid development has received considerable attention. In 1967, Fine reported a case of patient with multiple keloids

ranging from 5-20 years duration that all contained hair and sebaceous glands. Shortly after that it was demonstrated that while it was known that there was abundant acid phosphatase in the keratogenous zones of epidermis and hair follicles and in center of sebaceous glands, increased acid phosphatase activity was found in keloids (10x normal) and that there may be significant differences in the epidermal enzyme activity among different body regions (Im & Hoopes, 1971). In 1978, Osman, Gumma & Satir studied 84 keloid patients and 60 controls of both sexes in the Sudan. They demonstrated that the sections of keloid tissue all showed active hypertrophied sebaceous acini. Based on this they hypothesized the "Sebum Auto-immune Mechanism" in keloid formation which states that after injury a functioning sebaceous gland secretes sebum intradermally which acts as antigen initiating an auto-immune granulomatous response that may progress to keloid formation. It was felt that regional predilection of keloid is determined by the presence of sebaceous glands and evidence for this was felt to be that the palm, sole and lips are devoid of sebaceous glands and keloids (as opposed to HTS) have not been reported in these 3 areas. They also proposed that hormones (namely testosterone) stimulates acinar cells to secrete sebum and that when there is weak hormone stimulation and a weak auto-immune response occurs HTS may be formed instead of keloid. Finally, it was suggested that when keloids do not form in sebaceous gland containing areas the injury might not have detached acinar cells from their ductal systems.

Yagi, Dafalla & Osman (1979) skin tested 40 patients with keloids with an antigen made by homogenizing sebum in olive oil and injecting preparation intradermally (unfortunately, the authors did not state from whom they obtained the sebum - keloid formers or non-keloid formers). All 40 patients exhibited positive skin tests defined as a weal and/or swelling at least 5 mm in diameter. The authors then desensitized 11 patients with a positive skin tests with 6 sebum vaccines prior to

excision of their keloids and 2 post operative sebum vaccines and found that 1 year later only 1 out of 11 had recurrences.

Questions have been raised as to the uniqueness of sebum composition among patients. Stewart, Downing and Strauss (1982) studied scalp lipids of 10 males fractionated by Thin-Layer Chromatography and by High Performance Lipid Chromatography and found that sebum fatty acid composition varied widely between subjects especially in amounts of iso-branched acids and that individual composition is maintained even after profound reduction in sebum synthesis by 13 cis-retinoic acid. Green, Stewart, and Downing (1984) analyzed 4 sebum samples collected at 2 week intervals from 10 subjects by quartz capillary gas chromatography and found marked differences in wax ester fatty acid composition between subjects and a constancy of composition for each of the subjects over the 2 months which suggested that since the composition was constant for subjects but varied between subjects that sebum composition is under genetic control.

Serum factors have been hypothesized to play a role in keloid formation. Cohen et al. (1976) studied serum alpha-globulin which are inhibitors of collagenase activity and prevent collagen degradation and found that they occur in higher levels in keloidal and hypertrophic scars than in normal skin and scar. McCoy & Cohen (1981) performed a study to determine if sera from keloid or nonkeloid control patients could alter growth kinetics or collagen synthesis in keloid derived or normal dermal fibroblasts. They found that keloid sera did not alter relative collagen synthesis by either normal dermal or keloid derived fibroblasts compared with sera from age, sex and race-matched nonkeloid formers and that the cellular growth rates of keloid fibroblasts in both normal human and keloid sera were similar. From this it was concluded that sera from keloid patients did not contain factors that significantly modify in vitro growth kinetics or collagen synthesis of keloid derived or normal dermal fibroblasts.

TREATMENT MODALITIES FOR KELOIDS

While an in depth discussion of treatment of keloids is beyond the scope of this thesis the principal modalities of therapy for the problem of keloids will be briefly reviewed. Interested readers are referred to the references following each section. Basic reviews may be found in the papers of Kitlowski (1951), Ketchum et al. (1974), and Rudolph (1987).

Surgical excision is the most popular therapy (Cosman et al., 1961; Cosman and Wolff, 1972; Brown and Pierce, 1986). It alone is usually unsuccessful in the treatment of keloids since the same biomechanical and mechanical forces act on new wound as acted on the old. There have been cases of success reported with surgical intervention when a HTS is related to infection or due to the incision or laceration being perpendicular to relaxed skin tension lines.

Cryosurgery has been used in attempts to treat keloids (Shepherd and Dawber, 1982; Muti and Ponzio, 1983). This therapeutic intervention has been based on the fact that cold injury to normal skin results in less collagen deposition and less wound contraction than heat injury (Li et al., 1980). Cryosurgery results in macroscopic injury and tissue death and heal by second intention. The investigations of Shepherd and Dawber (1982) have demonstrated that keloids respond poorly to cryosurgery.

Many pharmacologic interventions have been reported. The most widely used appears to be glucocorticoids (Murray, 1963; Maguire, 1965; Ketchum et al, 1966; Griffith, 1966; Vallis, 1967; Griffith, et al., 1970 Converse and Stallings, 1972; Garcia-Velazco, 1972; Vallis, 1973; Singleton & Gross, 1973; Jaworski, 1974; Kyrtatas, 1974; Im et al., 1975; Bowszyc, 1977; Kiil, 1979; Eid, 1980). Intralesional and to a lesser extent topical administration of triamcinolone demonstrates consistent efficacy. It is believed that injection at time of excision acts as anti-inflammatory to retard the intensity of the wound healing response and that injection

into established keloids tips the balance from collagen anabolism to catabolism. Keloids and normal fibroblasts both have glucocorticoid receptors and while in normal fibroblasts glucocorticoids stimulate cellular proliferation and accelerate transport of the amino acids glycine and proline as well as decreasing collagen production, keloid fibroblasts are hyperresponsive with acceleration of amino acid transport (5 fold acceleration over normal fibroblasts) but are not stimulated to proliferate nor is collagen production inhibited (Russell et al., 1982; Gadson et al., 1984). Local anesthetics with glucocorticoids as dilutents are felt to inhibit collagen synthesis and secretion (Eichhorn & Peterkofsky, 1979). Drugs such as lidocaine, bupivacaine, mepivacaine, dibucaine, tetracaine, procaine and cocaine have all been used for this purpose.

Lathyrogens (ex. aminopropionitrile, BAPN, penicillamine) inhibit the extracellular event of intermolecular crosslinking of collagen by chelating copper ions making them unavailable for the copper containing enzyme lysyl oxidase which catalyzes the first reaction in crosslinking process (Peacock, 1981). They have been used locally in an attempt to prevent the recurrence of keloid formation but they have very limited systemic use since they globally inhibit collagen crosslinking leading to ligamentous attrition and injury and vascular aneurysms (Aren et al., 1979; Chvapil et al., 1981; Fleisher et al., 1981; Apeer et al., 1985).

The use of retinoids is currently under investigation. The role of retinoids as fibroblast modulators has been questioned. It is known that Vitamin A deficiency causes a qualitative increase in chondroitin sulfates in skin and that increased chondroitin sulfates are characteristic of keloids and HTS (Leela & Rama-Rao, 1970). Abertel et al. (1985) studied effects of the retinoids all-trans-retinoic acid and 13-cis-retinoic acid on the effects of procollagen production in keloid fibroblast cultures that were characterized by enhanced procollagen synthesis in vitro. It was demonstrated that there was a marked

decrease in collagen production in cultures treated with either of the retinoids and that the activity of prolyl hydroxylase was not affected while production of collagenase was reduced and the activity of an elastase like neutral protease was enhanced. It was concluded that there is a differential modulation of connective tissue metabolism by retinoids in keloid cell cultures and this suggests a possible new mode of pharmacologic treatment for keloids.

Colchicine has been used in the treatment of massive keloids (Peacock et al., 1970). It has been found to inhibit cellular secretion of newly synthesized procollagen by depolymerizing and disrupting the microtubular system required for transport of procollagen from the endoplasmic reticulum to the cell membrane. Vitamin E exhibits an anti-inflammatory effect by stabilizing lysosomal membranes and its effects are identical to that of glucocorticoids and has been used as pharmacologic therapy (Edgerton et al., 1951). Vitamin C (Ringsdorf & Cheraskin, 1982) and Madecassol (an antikeloid agent related to asiatic acid which is extracted from a plant called *Centella asiatica*) (Bosse, et al, 1980) have both been used in the treatment of keloids.

The third modality of keloid therapy includes all of the physical means to control keloid recurrence. Radiation was one of the earliest of these to be used (Levitt & Gillies, 1942; Nicoletis & Chassagne, 1968; King & Salzman, 1970; Garcez et al., 1974; Asakura et al, 1974; Levy et al, 1976; Ketchum, 1978). Radiation has been found to retard the growth of developing endothelial vascular buds as well as decrease the proliferation of new fibroblasts which decrease the amount of collagen produced (Levy et al., 1976). While this modality has been found effective the complications were numerous including hyperpigmentation, persistent erythema, telangiectasia, hypopigmentation, and atrophic skin changes. Pressure therapy has been attempted using elastic compression garments, spring pressure earrings and rigid facial masks (Del Maestro et al, 1967; Linares, 1975; Brent, 1978; Hurtado & Crowther, 1985).

The mechanism of this is uncertain and may revolve around increased tissue hypoxia, decreased edema, or decreased wound mast cells. Laser therapy using bioinhibition of fibroblast function based on Nd:YAG laser inhibition of collagen production by keloid derived fibroblasts in culture has been attempted with some success in the management of keloids and HTS (Apfelbert et al., 1985).

The final modality of therapy consists of a combination of two or more of above. Excision and radiotherapy have been attempted (Icochea & Rodriquez, 1970; Rodriquez de Lima and Antonio Vera, 1970; Malaker et al, 1976; Ollstein et al., 1981; Enhamre & Hammar, 1983). Perhaps the most common combination today is excision and steroid therapy (Converse & Stallings, 1972; Singleton & Gross, 1973; Shons & Press, 1983).

PURPOSE OF THIS STUDY

Although there have been several hypotheses put forth to explain keloid formation there has not been a great deal of definitive work on elucidating the predictive factors. Review of the literature on factors associated with keloid formation have thus far presented conflicting information as to the matter of ABO blood type. Just as ABO blood type association with keloid formation has been conflicting so has data concerning the predominance of any particular HLA histocompatibility antigen among keloid formers. As cited in this review of the literature age as well various medical conditions have been associated with keloid formation. In addition genetic predisposition has been implicated.

This thesis project was undertaken to investigate the possible association of: (1) any of the HLA A, B, Bw or Cw antigens (2) any of the ABO blood groups (3) any medical conditions (4) any family history of keloid formation or medical conditions (5) any drug or non-drug allergy (6) age at wounding (7) incidence surrounding ear piercing with keloid formation. These factors were investigated in two groups, patients with earlobe keloids (the only indisputable form of keloid) and a control group which were

free of any objectionable scars in an attempt to determine factors which would indicated either an increased risk of keloid formation or which might have a protective effect against keloid formation. Events associated with keloidal scarring, the average amount of time before the development of keloidal scarring, the proportion of keloid formers which formed multiple keloids and the locations of their keloids, as well as treatments which were attempted for the patients' keloids were also investigated in an effort to gain insight into the progression of keloid formation.

The results of this study indicated that there were differences in the frequencies of occurrence of several HLA-A, HLA-B, HLA-Bw, and HLA-Cw antigens among the two groups studied. This suggested that the possession of certain antigens conferred increased relative risk for keloid development while the possession of others appeared to have protective effects against keloid formation. It was also found that a positive history of several common medical conditions as well as a family history of keloid formation and other medical conditions similarly suggested either increased relative risk or protective effects in the formation of keloids. Differences in the distribution of ages in the two groups at the time of ear piercing was also found.

METHODS AND MATERIALS

EXPERIMENTAL SUBJECTS

Non-pregnant Black females between the ages of 15- and 63-years old with unilateral or bilateral earlobe keloids were invited to participate as subjects. Non-pregnant Black females between the ages of 15- and 63-years old who had never formed keloids were invited to participate as controls for the study. All subjects or their guardians signed an informed consent form outlining the experimental protocol which was approved by the Yale University School of Medicine's Human Investigations Committee (Protocol # 3896).

IDENTIFICATION OF POTENTIAL SUBJECTS

The Department of Surgical Pathology at Yale-New Haven Hospital keeps computerized records of all surgical specimens obtained from its operating rooms. By searching the files for the names of patients who had keloid specimens sent to pathology and reviewing the pathology report to elucidate from where the specimens were obtained, the names of all patients who had had earlobe keloids removed since 1983 were obtained. Review of patients' charts through the Department of Medical Records allowed telephone numbers of the patients to be obtained and potential subjects were contacted by telephone and invited to participate after gaining their physicians' approval.

SELECTION CRITERIA

Keloid-Former Subjects

Five criteria were used for inclusion as a keloid patient in this study:

(1) Sex - all patients were female. Only female patients were used so that questions of hormonal influence of keloids in females versus males would not

confuse the results of the study. This criterion was also imposed to help create a homogeneous sample for study.

(2) Age - all patients had to be over the age of 15 years old.

(3) Ethnic Background - only Black females were used as subjects of this study. HLA antigens have varied frequencies of occurrence in different ethnic persuasions. In order to elucidate the frequency of HLA antigens in a homogeneous group of keloid formers, all Black women were chosen. In addition, it has been reported that keloids occur with increasing frequency in people of darker pigmentation (Koonin, 1964; Oluwasanmi, 1974; Doyle & Passey, 1975; Morgan, Gilchrest, and Goldwyn, 1975), so that the largest population of patients with earlobe keloids were Black.

(4) Medical condition - patients who had any medical condition which would not allow them to participate due to inability to comply with instructions were excluded.

(5) Region of Keloid - only patients who had earlobe keloids were selected for this study. This criterion was imposed so that there could be not confusion as to whether the lesion was a keloid or a hypertrophic scar. It is impossible for a lesion exceeding the boundaries of the wound to be a hypertrophic scar and since the wound in the case of the earlobe keloid is a tiny puncture site any lesion which extends beyond it is by definition a keloid. It was due to the stringency of this criterion that the number of patients studied was small.

Control Subjects

Subjects who were asked to serve as controls for this study had to meet the first four of the above criteria. A mandatory criterion was that they had undergone ear piercing at least one year prior to the study and had not exhibited keloids at piercing site. Furthermore, they also had to be free of any other keloids at the time of the study.

Patients who possessed any questionable scar was categorically excluded from the study to ensure a control population which was free of any keloid formers.

DATA COLLECTION

SUBJECT HISTORY

All subjects were asked to complete a questionnaire which elicited the following information:

(1) Age

(2) Age at Ear Piercing - studies have indicated that there is an inverse relationship between age and keloid development (Alhady & Sivanantharajah, 1969; Ketchum et al., 1974; Ramakrishnan, 1974; Doyle & Passey, 1975)

(3) Keloid History including the occurrence of infections at the sight of ear piercing, multiple keloids and their locations - Investigators have commented on the regional distribution of keloids in the past (Koonin, 1964; Crockett, 1964; Alhady and Sivanantharajah, 1969). As noted earlier local wound infection can prolong the inflammatory phase of wound healing and retard wound healing (Reed & Clark, 1985).

(4) Past Medical History including an Allergy History - studies had indicated that states of infection might be associated with keloid formation (Koonin, 1964) and that various disease states can cause aberrant wound healing (Carrico et al., 1974; Reed & Clark, 1985)

(5) Previous Surgery - to assess patients' exposure to potentially keloid forming situations in addition to ear piercing

(6) Previous Lacerations- to assess patients' exposure to potentially keloid forming situations

(7) Family Keloid and Medical History - to ascertain any patterns of genetic predisposition which had been postulated in the literature (Koonin, 1964; Ramakrishnan, 1974)

A sample of the questionnaire can be found in the Appendix A.

BLOOD STUDIES

Blood Collection

13 ml of blood was collected from each subject at the time of encounter by aseptic venipuncture technique (Pendergraph, 1984). 10 ml was collected in a green top Vacutainer tube containing heparin which was later used for HLA histocompatibility testing. 3 ml was collected in a red top non-heparinized Vacutainer tube for ABO/Rh typing.

ABO/Rh Blood Group Typing

In order to assess the association of any particular ABO and Rh blood type with earlobe keloids blood samples from subjects in both the keloid-formers and control group were sent to the Yale-New Haven Hospital Blood Bank for typing. The blood samples were there typed by the method outlined in the Procedural Manual of the Blood Bank of Yale-New Haven Hospital (1988). The typing was carried out as follows:

Patients' blood groups were directly determined by reaction with sera containing antibodies to the A antigen and B antigen by a method known as Forward Typing. In this technique the patients' red blood cells are mixed with commercially obtained Blood Grouping Serum containing antibodies to the A antigen and B antigen. The mixtures are then inspected for agglutination which indicates the presence of the respective antigen. If both mixtures agglutinate blood group AB is indicated and if neither agglutinate blood group O is indicated. All apparent Group O blood was confirmed by adding the patient's red blood cells with commercially obtained Blood Grouping Serum containing antibodies to both the A and the B antigen and inspecting for agglutination. Agglutination of this serum in the presence of prior agglutination of both the Anti-A and Anti-B sera confirmed blood grouping O.

Results were checked by a procedure known as Back Typing in which the serum of the blood under investigation is tested for expected antibodies with known group A and group B red blood cells. In this technique the patient's serum is mixed with commercially obtained Red Blood Cells of a known ABO blood group type. The mixtures were inspected for agglutination. If the A cell and serum solution agglutinated in the absence of B cell and serum solution agglutination blood group B was confirmed. If B cell and serum solution agglutinated in the absence of A cell and serum solution agglutination blood group A was confirmed. If both the A and B cell and serum solutions agglutinated blood type O was confirmed. If neither the A cell nor the B cell and serum solutions agglutinated blood type AB was confirmed.

Rh type was ascertained by a technique in which the patient's red blood cells were added to first a commercially obtained serum containing IgG antibodies specific for the D antigen and then to the patient's own serum. The mixtures were inspected macroscopically for agglutination. If there was agglutination in the tube containing the Anti-D serum and the tube containing the patient's serum did not agglutinate the patient was considered Rh positive. If there was an absence of agglutination in both of the tubes the patient was considered Rh negative.

HLA Histocompatibility Antigen Typing

To determine the association of HLA histocompatibility antigens to the formation of earlobe keloids blood samples were obtained from all patients in this study as outlined above. The samples were then typed for HLA histocompatibility antigens according to the following complement-dependent microlymphocytotoxicity technique. In this procedure, viable lymphocytes were incubated with specific antisera and complement. If antigens present on the cell surface corresponded to the known antibodies in the sera complement-mediated cell lysis occurred. Cells were then stained with eosin dye. Live

cells excluded eosin while dead cells did not. This procedure employed in this study was developed by Teraski (1980) and is as follows:

Lymphocyte Separation

Peripheral blood lymphocytes were separated from the heparinized whole blood samples using a single step density gradient centrifugation technique reported by Boyum (1968). A commercially available density gradient solution was employed (Lymphocyte Separation Medium [LSM], Litton Bionetics, Charleston, SC). LSM is a sterile solution of a sucrose polymer, Ficoll®, and diatrizoate salts with a density of 1.077-1.080 at 20° C. 5-7 ml of heparinized blood was layered on top of 2.5 ml of LSM in a 15 ml centrifuge tube which was then centrifuged at approximately 400 x G for 40 minutes. Following centrifugation, substances in the tube layered as follows: the top yellow layer contained plasma and platelets; the second cloudy layer contained lymphocytes and platelets; the third clear layer contained LSM; and the fourth layer at the bottom of the tube contained red blood cells and granulocytes. Lymphocytes were pipetted from the tube and distributed into three 1.5 ml microcentrifuge tubes. Cells were twice washed by resuspending in a small amount of Phosphate Buffered Saline containing 1% Penicillin/Streptomycin and centrifuging at a speed of 300 x G for 10 minutes. Following washing and recovery, the cells were resuspended in RPMI 1640 medium with 25 mM Hepes (Irvine Scientific, Santa Ana, CA) containing 1% Penicillin/Streptomycin.

Lymphocyte Cell Density

Best results were achieved with a lymphocyte density of 1.8-2.0 x 10⁶ lymphocytes per ml of RPMI medium. Cells were counted using a Standard Improved Neubauer Hemacytometer counting chamber at 100x and diluted to a final concentration of 2.0 x 10⁶ cells per ml.

Microlymphocytotoxicity Technique For HLA Antigen Typing

Two types of HLA tissue typing trays were used to define the HLA-A, HLA-B, HLA-Bw and HLA-Cw locus antigens. A table of the HLA Class I (A, B, and C) Nomenclature can be found in Appendix B. The tray used was a Terasaki Second HLA-ABC 72 well typing tray (One Lambda Inc, Los Angeles, CA). Antigens specified on this tray can be found in Appendix C. Each well contained 1 µl of a specified HLA antisera and a positive and negative control dispensed onto a 72 microtiter plate. The second tray used was the Terasaki Black HLA-ABC 60 well tray (One Lambda Inc). The antigens specified on this tray can be found in Appendix D. This was a 60 well tray containing antisera to define antigens occurring with higher frequencies in Black populations as well as positive and negative controls. Trays were stored at -70° C until just prior to lymphocyte plating. For use trays were thawed at room temperature for 5-10 minutes. 1 µl of the lymphocyte suspension was added to each of the antisera wells using a Hamilton syringe attached to a repeating dispenser. Trays were incubated at room temperature for 30 minutes without agitation. Following incubation, 5 µl of rabbit complement (One Lambda Inc) was added to each well of the trays. The trays were then incubated at room temperature for 1 hour. Following this second incubation 5 µl of 5% (W/V) eosin was

added to each well followed approximately 2 minutes later by 10 μ l of 37% formalin as a fixative. To prevent desiccation each well was overlain with parafin oil.

Microscopic Evaluation Of Tests

Trays were scored with an inverted phase contrast microscope at 100x. Living cells excluded the eosin and appeared small and refractile. Dead lymphocytes stained with eosin and appeared larger, flatter, and darker. Death of the lymphocytes in a well indicated that the lymphocytes possessed the HLA antigen to the antisera contained in the well. Six scores were possible based on the percent of dead lymphocytes in each well in comparison to the negative control well.

<u>Score</u>	<u>Interpretation</u>	<u>% Dead Cells</u>
1	Negative	0-10
2	Doubtful negative	11-20
4	Weak positive	21-50
6	Positive	51-80
8	Strongly positive	81-100
0	Not readable	

STATISTICAL ANALYSIS OF DATA

The study was designed as a retrospective case-control study whose purpose was to generate hypotheses concerning ABO blood group type, HLA histocompatibility type, past medical history and family history and the outcome of keloid formation. To accomplish this a sample size of twenty (20) was obtained for each group (the keloid-former group and the control group). This size was determined by the available population of patients with unilateral or bilateral earlobe keloids who had received treatment at Yale-New Haven Hospital since 1983.

Demographic characteristics of the keloid-former and control groups were compared using the inner 95-percentile range (which is the range in which 95% of observed values fall), the median, and mean of patients' age at time of study and at time of ear piercing. All other data collected were analyzed using the software package StatWorks: Statistics with Graphics for the Macintosh from the Cricket Software Company, Data Metrics, Inc with the statistical advice of Dr. Robert Jacobson. Dr. Jacobson is a Fellow of the Robert Wood Johnson Clinical Scholars Program in the Department of Internal Medicine and a member of the Thesis Consulting Service. A sample of the coding sheet used can be found in Appendix E.

ABO blood group; HLA histocompatibility antigen; past medical history including history of drug, non-drug allergies, and infection after ear piercing; and family keloid and medical history data were analyzed using Cross-Tabulation analysis (generation of 2 x 2 tables). Each variable, eg. HLA-A1, was tabulated for its absence or presence across the two groups, keloid-formers and controls. An odds ratio was calculated for each comparison. As has been shown in the literature, the odds ratio is an excellent approximation of relative risk and was used as such in this study (Feinstein, 1985). Relative risk can be used to measure the strength of an association (Wynder et al., 1982). In this calculation, in order to avoid the problem of potentially dividing by zero, the value of 0.5 was added to each cell in any table generated by the Cross Tabulation analysis which contained a cell with a value of zero. Quantitative significance between the groups was to be considered an odds ratio of greater than 2.000 (an indication of increased relative risk), or less than 0.500 (an indication of decreased relative risk or the factor having a protective effect) (Hutchison, 1968; Wynder et al., 1982; Wynder, 1987).

An advantage of using the odds ratio to assess association is that calculation of this statistic is not affected by the sample size as long as the samples are true representatives of the populations investigated (Feinstein, 1985). This allowed

conclusions to be drawn from the data even though the sample sizes were relatively small because stringency of inclusion criteria ensured that patients in the keloid group truly had keloids and those in the control group were free of keloidal scars.

Nonparametric comparisons were made between the keloid-former and control groups using the Chi-Square test, and its associated level of significance was calculated to assess the stability of the odds ratios. Although the traditional level of significance is 0.05, due to the small sample size and large number of variables assessed, we chose a less stringent level of significance of 0.10 in order to identify possible associations. It is realized that definitive conclusions cannot be drawn at this level of significance, but it allowed the separation of variables which can be used later as bases of investigation.

RESULTS

Demographic Characteristics of Keloid and Control Groups

The demographic characteristics of the groups studied can be found in Table 1. Twenty women with earlobe keloids participated in the research protocol. ABO blood group typing and HLA histocompatibility typing were performed on samples of blood obtained from all members of the keloid group. All 20 completed the questionnaire. The median, mean, and inner 95-percentile age range at examination of this group were 31 years, 34.30 years, and 18-62 years respectively. The median, mean and inner 95-percentile age ranges at ear piercing for this group were 17 years, 20.58 years, and 9-35 years respectively.

HLA histocompatibility typing was performed on 20 of the patients in the control group. ABO blood group typing was performed on samples of blood obtained from 23 of the patients in this group and the questionnaire was also filled out by 23 women in this group. The median, mean, and inner 95-percentile age range at examination of this group were 26 years, 27.26 years, and 22-49 years, respectively. The median, mean, and inner 95-percentile age range at ear piercing in the control group were 4 years, 8.54 years, and 1-22 years respectively.

Table 1. Demographic Characteristics of the Keloid and Control Groups

	<u>Keloid</u>	<u>Control</u>
Number of Patients		
ABO Blood Group Data	20	23
HLA Histocompatibility Antigen Data	20	20
Questionnaire	20	23
Age on Examination		
Inner 95-percentile age range	18-62 years	22-49 years
Median age	31 years	26 years
Mean age	34.30 years	27.26 years
Age At Time of Ear Piercing		
Inner 95-percentile age range	9-35 years	1-22 years
Median age	17 years	4 years
Mean age	20.58 years	8.54 years

Interpretation of Data

Data was interpreted as outlined in the Statistical Analysis section of the Methods and Materials section. By way of summary: (1) Variables present in higher frequencies in the keloid-former group which were considered clinically significant had odds ratios (o) greater than or equal to 2.000. Since odds ratios approximate relative risk ratios, these odds ratios were considered to suggest increased relative risk in the development of keloid formation. (2) Variables present in higher frequencies in the control group which were considered clinically significant had odds ratios of less than or equal to 0.500. These odds ratios were considered to have protective effects against the development of keloids. In all cases statistical significance was considered $p \leq 0.10$.

ABO/Rh Blood Group Data

ABO blood groups were defined as outlined above. The results of the analysis are shown in Table 2. As can be seen, the following blood groups were not encountered in either group: A negative, B negative and AB negative. The presence of the type AB positive was found with greater frequency in the control group and was considered clinically significant ($o = 0.358$) indicating a protective effect. This result, however, was not found to be statistically significant.

Table 2. Odds ratio, Chi-Square, and level of significance analysis of the ABO Blood Group Data Obtained from the Keloid and Control Groups.

<u>Blood Group</u>	<u>Number</u>		<u>Odds Ratio</u>	<u>Chi-Square</u>	<u>Significance (p)</u>
	<u>Keloid</u>	<u>Control</u>			
O POSITIVE	8	11	0.727	0.266	0.606
O NEGATIVE	1	1	1.158	0.010	0.919
A POSITIVE	8	7	1.524	0.431	0.512
A NEGATIVE*	0	0	1.146	0.000	1.000
B POSITIVE	3	3	1.176	0.034	0.853
B NEGATIVE*	0	0	1.146	0.000	1.000
AB POSITIVE*	0	1	0.358	0.890	0.345
AB NEGATIVE*	0	0	1.146	0.000	1.000

*odds ratio calculated by adding 0.5 to each cell to avoid problem of cells containing zeros.

Expected vs. Observed Incidences of the Most Commonly Occurring HLA-A and HLA-B Antigens Among Blacks

In order to assess how representative the control sample was of the Black population the expected incidences of the HLA-A and HLA-B antigens occurring most commonly in Blacks (as reported in Histocompatibility Testing 1980 Appendix F) was compared to the observed incidences in this study (Table 3). In the case of narrow specificities such as Aw 23, Bw 44 and Bw 58, the data was corrected for their broad specificities A9, B 12 and B 17 respectively. This table allows the reader to note the variation in this control group with the one published set of data. As can be seen from this table the incidences of occurrence of the major HLA-A and HLA-B antigens in the control group can be considered as a representative of the Black population at large and serves as a valid reference standard against which we can compare the keloid group.

Table 3. Expected vs. Observed Incidences in the Control Group of the Most Commonly Occurring HLA-A and HLA-B Histocompatibility Antigens in Blacks.

ANTIGEN	EXPECTED INCIDENCE	OBSERVED INCIDENCE
A 2	27.3	35
A 3	14.3	15
AW 23 (9) corrected for A9	20.4	15
A 28	16.6	20
A 29	12.3	30
Aw 34	12.5	0
B 7	17.0	20
Bw 35	12.1	25
Bw 42	14.8	5
Bw 44 (12) corrected for B12	13.7	15
Bw 53	12.6	20
Bw 58 (17) corrected for B17	20.3	35

HLA Histocompatibility Antigen Data

Women from both groups were typed for the 24 known HLA-A antigens and results of this analysis are summarized in Table 4. Of the 24 known antigens, 6 were not found to be present in any patient in either group and these included A 32, Aw 74, Aw 68, Aw 69, Aw 36, and Aw 43.

HLA-A2 was found to occur in greater frequency in the control group with a clinically and statistically significant ($p = 0.058$) odds ratio indicating a protective effect. Three of the HLA-A antigens present with a greater frequency in the keloid population were found to have statistically significant odds ratios indicating a positive association between the antigen and keloid formation. These antigens included: HLA-A 9 ($p=0.038$), HLA-A 23 (a narrow specificity A 9) ($p=0.058$), and HLA-Aw 34 ($p=0.072$).

Four other HLA-A antigens which occurred with greater frequency in the control population were found to have clinically significant odds ratios. HLA-A1 ($o=0.211$), A 25 ($o=0.317$), A 26 ($o=0.317$), and Aw 66 ($o=0.474$) were found to have protective odds ratios. None of these results, however, reached statistical significance.

Table 4. Odds ratio, Chi-Square, and level of significance analysis of the HLA-A Histocompatibility Antigen Data Obtained from the Keloid and Control Groups.

<u>HLA-A Antigen</u>	<u>Number</u>		<u>Odds Ratio</u>	<u>Chi-Square</u>	<u>Significance (p)</u>
	<u>Keloid</u>	<u>Control</u>			
A 1	1	4	0.211	2.057	0.151
A 2	2	7	0.206	3.584	0.058
A 3	2	3	0.630	0.229	0.633
A 9	9	3	4.636	4.286	0.038
A 23	7	2	4.846	3.584	0.058
A 24	2	1	2.111	0.360	0.548
A 10	4	5	0.750	0.143	0.705
A 25*	0	1	0.317	1.026	0.311
A 26*	0	1	0.317	1.026	0.311
Aw 34*	3	0	8.200	3.243	0.072
Aw 66	1	2	0.474	0.360	0.548
Aw 11	2	2	1.000	0.000	1.000
Aw 19	9	8	1.227	0.102	0.749
Aw 29	2	1	2.111	0.360	0.548
A 30	5	6	0.778	0.125	0.723
A 31	1	1	1.000	0.000	1.000
A 32*	0	0	1.000	0.000	1.000
A 33	3	2	1.588	1.229	0.633
Aw 74*	0	0	1.000	0.000	1.000
A 28	5	4	1.333	0.143	0.705
Aw 68*	0	0	1.000	0.000	1.000
Aw 69*	0	0	1.000	0.000	1.000
Aw 36*	0	0	1.000	0.000	1.000
Aw 43*	0	0	1.000	0.000	1.000

*odds ratio calculated by adding 0.5 to each cell to avoid problem of cells containing zeros

Of the fifty known HLA-B antigen types defined, twenty-one were not found in either group as shown in Table 5. Six were found in greater frequency in the control group and reached clinical significance indicating a protective effect. These included: B 8 (o=0.317), B 16 (o=0.474), B 17 (o=0.328), Bw 57 (o=0.474), Bw 22 (o=0.317), and B 37 (o=0.180). Twelve were found to occur in greater frequency in the keloid group with clinically significant odds ratios indicating a increased risk of keloid formation. These antigens included: B 5 (o=2.111), B 51 (o=2.111), B 44

($o=2.111$), B 13 ($o=3.154$), B 14 ($o=2.111$), Bw 62 ($o=2.111$), B 18 ($o=5.541$), B 21 ($o=3.154$), B 49 ($o=3.145$), B 40 ($o=5.541$), Bw 60 ($o=5.541$), and Bw 48 ($o=3.154$). In neither group did any of these odds ratios reach statistical significance.

Table 5. Odds ratio, Chi-Square, and level of significance analysis of the HLA-B Histocompatibility Data Obtained from the Keloid and Control Groups.

<u>HLA-B Antigen</u>	<u>Number</u>		<u>Odds Ratio</u>	<u>Chi-Square</u>	<u>Significance (p)</u>
	<u>Keloid</u>	<u>Control</u>			
B 5	2	1	2.111	0.360	0.548
B 51	2	1	2.111	0.360	0.548
Bw 52*	0	0	1.000	0.000	1.000
B 7	3	4	0.706	0.173	0.677
B 8*	0	1	0.317	1.026	0.311
B 12	4	3	1.417	0.173	0.677
B 44	2	1	2.111	0.360	0.548
B 45	0	0	1.000	0.000	1.000
B 13*	1	0	3.154	1.026	0.311
B 14	2	1	2.111	0.360	0.548
Bw 64*	0	0	1.000	0.000	1.000
Bw 65*	0	0	1.000	0.000	1.000
B 15	3	2	1.588	0.229	0.633
Bw 62	2	1	2.111	0.360	0.548
Bw 63*	0	0	1.000	0.000	1.000
Bw 75*	0	0	1.000	0.000	1.000
Bw 76*	0	0	1.000	0.000	1.000
Bw 77*	0	0	1.000	0.000	1.000
B 16	1	2	0.474	0.360	0.548
B 38*	1	1	1.000	0.000	1.000
B 17	3	7	0.328	2.133	0.144
Bw 57	1	2	0.474	0.360	0.548
Bw 58*	0	0	1.000	0.000	1.000
B 18*	2	0	5.541	2.105	0.147
B 21*	1	0	3.154	1.026	0.311
B 49*	1	0	3.145	1.026	0.311
Bw 50*	0	0	1.000	0.000	1.000
Bw 22*	0	1	0.317	1.026	0.311
Bw 54*	0	0	1.000	0.000	1.000
Bw 55*	0	0	1.000	0.000	1.000
Bw 56*	0	0	1.000	0.000	1.000
B 27*	0	0	1.000	0.000	1.000
B 35	4	5	0.750	0.143	0.705
B 37*	0	2	0.180	2.105	0.147
B 40*	2	0	5.541	2.105	0.147
Bw 60*	2	0	5.541	2.105	0.147
Bw 61*	0	0	1.000	0.000	1.000
Bw 41	1	1	1.000	0.000	1.000

Bw 42	1	1	1.000	0.000	1.000
Bw 46*	0	0	1.000	0.000	1.000
Bw 47*	0	0	1.000	0.000	1.000
Bw 48*	1	0	3.154	1.026	0.311
Bw 53	5	4	1.333	0.143	0.705
Bw 59*	0	0	1.000	0.000	1.000
Bw 67*	0	0	1.000	0.000	1.000
Bw 70	2	2	1.000	0.000	1.000
Bw 71	2	2	1.000	0.000	1.000
Bw 72*	0	0	1.000	0.000	1.000
Bw 73*	0	0	1.000	0.000	1.000

*odds ratio calculated by adding 0.5 to each cell to avoid problem of cells containing zeros.

Results from analysis of the HLA Public Specificities Bw4 and Bw 6 are shown in Table 6. HLA-Bw 6 was found to occur in greater frequency in the keloid population reaching clinical significance ($o=3.857$). This suggested increased risk of keloid formation in patients with this antigen. This result, however, did not reach statistical significance.

Eight of the eleven known HLA-Cw antigens were analyzed and results are summarized in Table 7. HLA-Cw2 occurred with greater frequency in the keloid population and was both clinically significant ($o=6.333$) (indicating increased risk) and statistically significant ($p=0.077$). HLA-Cw 3 ($o=3.350$) and Cw 5 ($o=3.350$) occurred in greater frequency in the keloid population and were found to have clinically significant odds ratios indicating increased risk. However, these results were not found to be statistically significant. HLA-Cw 1 occurred with greater frequency in the control group suggesting a protective effect against keloid formation ($o=0.180$), but, this result did not reach statistical significance. Only the HLA-Cw 8 antigen was found in neither group of patients.

Table 6. Odds ratio, Chi-Square, and level of significance analysis of the HLA Public Specificities Data Obtained from the Keloid and Control Groups.

<u>HLA-Bw Antigen</u>	<u>Number</u>		<u>Odds Ratio</u>	<u>Chi-Square</u>	<u>Significance (p)</u>
	<u>Keloid</u>	<u>Control</u>			
Bw 4	12	13	1.556	0.107	0.744
Bw 6	18	14	3.857	2.500	0.114

Table 7. Odds ratio, Chi-Square, and level of significance analysis of the HLA-Cw Histocompatibility Antigen Data Obtained from the Keloid and Control Groups.

<u>HLA-Cw Antigen</u>	<u>Number</u>		<u>Odds Ratio</u>	<u>Chi-Square</u>	<u>Significance (p)</u>
	<u>Keloid</u>	<u>Control</u>			
Cw 1*	0	2	0.180	2.105	0.147
Cw 2	5	1	6.333	3.137	0.077
Cw 3	3	1	3.350	1.111	0.292
Cw 4	9	9	1.000	0.000	1.000
Cw 5	3	1	3.350	1.111	0.292
Cw 6	2	3	0.630	0.229	0.633
Cw 7	2	3	0.630	0.229	0.633
Cw 8*	0	0	1.000	0.000	1.000

*odds ratio calculated by adding 0.5 to each cell to avoid problem of cells containing zeros

Table 8 summarizes all of the HLA-A, HLA-B, HLA-Bw, and HLA-Cw which achieved statistical significance along with their implications. A comparison of the numbers of these antigens found between the keloid and control groups is located in Appendix G.

Table 8. HLA Histocompatibility Antigens Reaching Statistical Significance and Their Effect.

<u>Antigen</u>	<u>p</u>	<u>Effect</u>	
		<u>Protective</u>	<u>Increased Risk of Keloid Formation</u>
A 2	0.058	+	
A 9	0.038		+
A 23 (9)	0.058		+
Aw 34	0.072		+
Cw 2	0.077		+

Past Medical History Data

Information was obtained from both groups pertaining to their past medical history with respect to 17 common medical conditions and the results of the analysis are shown in Table 9. Of the 17 conditions under investigation no patient in either group reported a positive history of tuberculosis, epilepsy or kidney disease. Among those conditions reported only a positive history of hypertension, occurring in greater frequency in the keloid population, was found to reach both clinical significance ($\alpha=9.400$) (indicating increased risk of keloid formation) and statistical significance ($p=0.054$). Six conditions were found to occur in greater frequency in the keloid group indicating an increased risk. These conditions included heart disease ($\alpha=3.615$), blood disease ($\alpha=2.333$), lung disease ($\alpha=3.615$), arthritis ($\alpha=3.615$), uterine disease ($\alpha=3.882$), and a subjective sense of easy bleeding ($\alpha=6.351$). Two conditions were

found which occurred in greater frequency in the control group and thus had clinically protective odds ratios. These were a positive history of cancer ($o=0.366$), and a positive history of diabetes ($o=0.345$). However, these results did not reach statistical significance.

The results of the analysis of patient reported history of allergies are summarized in Table 10. Only a history of allergies to substances other than drugs (including pollen, dogs, cats, rodents, dust, shellfish, chocolate, citrus fruits) was found to be both statistically significant ($p=0.100$) and clinically significant ($o=0.327$). This occurred in greater frequency in the control group indicating a protective effect against keloid formation. Allergies to penicillin ($o=0.250$) and sulfa drugs ($o=0.210$) occurred in increased frequency in the control population and were found to be clinically significant, but did not reach statistical significance. A history of allergy to aminoglycosides was found to occur in greater frequency in the keloid group with a clinically significant odds ratio ($o=3.615$) indicating increased relative risk. This result was not statistically significant.

A positive history of infection after ear piercing occurred in greater frequency in the keloid group. This was found to have both statistical significance ($p=0.067$) and clinical significance ($o=6.667$). This result indicated an increased risk of keloid formation and is summarized in Table 11.

Table 9. Odds ratio, Chi-Square, and level of significance analysis of the Past Medical History Data Obtained from the Keloid and Control Groups.

<u>Patient Disease</u>	<u>Number</u>		<u>Odds Ratio</u>	<u>Chi-Square</u>	<u>Significance (p)</u>
	<u>Keloid</u>	<u>Control</u>			
Tuberculosis*	0	0	1.146	0.000	1.000
Cancer	0	1	0.366	0.890	0.345
Diabetes	0	1	0.366	0.890	0.345
Epilepsy*	0	0	1.146	0.000	1.000
Heart Disease*	1	0	3.615	1.177	0.278
Blood Disease	2	1	2.444	0.527	0.468
Asthma	2	3	0.741	0.096	0.756
Lung Disease*	1	0	3.615	1.177	0.278
Kidney Disease*	0	0	1.146	0.000	1.000
GI Disease	1	2	0.553	0.225	0.635
Arthritis	1	0	3.615	1.177	0.278
Skin Disease	2	2	1.167	0.022	0.883
Hypertension*	3	0	9.400	3.709	0.054
Uterine Disease	3	1	3.882	1.439	0.230
Chronic Infections	1	3	0.351	0.820	0.365
Easy Bleeding	2	0	6.351	2.412	0.120
Easy Bruising	3	2	1.853	0.414	0.520

Table 10. Odds ratio, Chi-Square, and level of significance analysis of the Patient's History of Drug and Non-Drug Allergies Data Obtained from the Keloid and Control Groups.

<u>Allergy</u>	<u>Number</u>		<u>Odds Ratio</u>	<u>Chi-Square</u>	<u>Significance (p)</u>
	<u>Keloid</u>	<u>Control</u>			
Drug Allergies					
Penicillin	1	4	0.250	1.598	0.206
Aminoglycosides	1	0	3.615	1.177	0.278
Sulfa Drugs	0	2	0.210	1.824	0.177
Non-Drug Allergies	2	7	0.327	2.699	0.100

*odds ratio calculated by adding 0.5 to each cells to avoid problem of cells containing zeros

Table 11. Odds ratio, Chi-Square, and level of significance analysis of the Infection after Ear Piercing Data Obtained from the Keloid and Control Groups.

<u>Infection</u>	<u>Number</u>		<u>Odds Ratio</u>	<u>Chi-Square</u>	<u>Significance (p)</u>
	<u>Keloid</u>	<u>Control</u>			
Infection	5	1	6.667	3.359	0.067

Family History Data

Family history of keloid formation and family history of 10 common medical conditions were obtained from both groups and the results of the analysis of these data can be found in Tables 12 and 13 respectively. Patients in the keloid groups reported a positive family history of keloid formation with greater frequency than patients in the control group indicating an increased risk in keloid formation. Clinically significant odds ratios were found for the reports of a positive history of keloid formation in a father ($o=3.615$), a sister ($o=2.500$), or any relative ($o=2.036$). While these indicate increased risk for keloid development, these results did not reach statistical significance.

A positive family history of tuberculosis was reported more frequently in the control group suggesting a clinically significant protective effect ($o=0.143$). This result achieved statistical significance ($p=0.094$). A positive family history of epilepsy occurred more frequently in the keloid group achieving clinical significance ($o=2.444$) which indicated increased risk for keloid formation. However, this result was not statistically significant. A positive family history of cancer, hypertension and blood disease were reported more frequently in the control group. These results achieved clinically significant odds ratios of 0.667, 0.450, and 0.269, respectively,

suggesting a protective effect. However, these results did not reach statistical significance.

Table 12. Odds ratio, Chi-Square, and level of significance analysis of the Family History of Keloid Formation Data Obtained from the Keloid and Control Groups.

<u>Family Member</u>	<u>Number</u>		<u>Odds Ratio</u>	<u>Chi-Square</u>	<u>Significance (p)</u>
	<u>Keloid</u>	<u>Control</u>			
Mother	2	2	1.167	0.022	0.883
Father*	1	0	3.615	1.177	0.278
Brother	2	1	1.625	0.146	0.702
Sister	2	1	2.500	0.536	0.464
Children	1	0	1.080	0.327	0.567
Any Relative	6	4	2.036	0.953	0.329

Table 13. Odds ratio, Chi-Square, and level of significance analysis of the Family Medical History Data Obtained from the Keloid and Control Groups.

<u>Disease</u>	<u>Number</u>		<u>Odds Ratio</u>	<u>Chi-Square</u>	<u>Significance (p)</u>
	<u>Keloid</u>	<u>Control</u>			
Tuberculosis	0	3	0.143	2.804	0.094
Cancer	6	9	0.667	0.393	0.531
Diabetes	11	12	1.120	0.034	0.853
Epilepsy	2	1	2.444	0.527	0.468
Heart Disease	5	9	0.519	0.973	0.324
Hypertension	15	20	0.450	1.010	0.315
Blood Disease*	0	2	0.269	1.824	0.177
Asthma	8	6	1.889	0.943	0.331
Lung Disease	0	0	1.146	0.000	1.000
Kidney Disease	2	3	0.741	0.096	0.756

*odds ratio calculated by adding 0.5 to each cell to avoid problem of cells containing zeros

Keloid Progression Data

The twenty women in the keloid formers group were asked to recall any incident involving their earlobes between the time of ear piercing and the initial onset of keloid formation. The results of the analysis of this data are summarized in Table 14. The group reported one of three occurrences: 55% of the group reported no event occurring, 25% reported infection, and 20% reported injury to their ear (including trauma secondary to pulling of earrings and human bites to the ear).

This same group was asked to recall the interval of time which between ear piercing and the onset of keloid formation (Table 14). The majority of these women (55%) reported an interval of greater than one year. Twenty percent of these women reported intervals less than one month. The remaining 25% indicated intervals ranging from greater than one month to less than one year.

Table 14. Analysis of the Keloid Progression Data Obtained from the Keloid Group.

	Number	Percentage
Total Number of Keloid Patients	20	100
Reported Events Associated with Keloid Formation		
No significant event	11	55
Injury	4	20
Infection	5	25
Time Noted Between Piercing and Onset of Keloid Formation		
Less than 1 month	4	20
1 - 3 months	1	5
3 - 6 months	2	10
6 - 9 months	1	5
9 months - 1 year	1	5
Greater than 1 year	11	55

Multiple Keloid Data

While the control group was questioned and found to be completely free of any objectionable scars, there were women in the keloid group who reported a number of objectionable scars and their distribution (Table 15). 65% of the keloid formers had multiple keloids. The majority of these keloids occurred on the patients abdomens (36.4%) with the next most highest location being on the upper extremity (31.85). None of the women in this group reported having keloids anywhere on there heads.

Table 15. Analysis of the Percentage of Multiple Keloids and their Locations Data Obtained from the Keloid Group.

	Number	Percentage
Total Number of Keloid Patients	20	100
Number of Keloid Patients with Multiple Keloids	13	65
Total Number of Other Keloids Reported	22	100
Location of Other Keloids		
Head	0	0
Neck	3	13.6
Chest	2	9.1
Abdomen	8	36.4
Upper Extremity	7	31.8
Lower Extremity	1	4.5
Back	1	4.5

Therapeutic Efficacy

While the goal of this study was to address the risk factors for keloid formation, the opportunity was taken to question keloid patients about any therapeutic interventions which they had attempted and their efficacy. Fourteen of the 20 women in this group reported some form of prior therapy. For the purposes of this study a failed outcome was considered a recurrence of the keloid, while a successful outcome had no recurrence. Of the 6 women who reported having surgical excision alone only 1 out the 6 reported a successful outcome. Three women reported local steroid injections alone and all 3 reported failure of this. Ten women reported the use of surgical excision and local steroid injection and all 10 reported successful outcomes. One woman reported radiation alone which failed. Four women reported attempting surgical excision and radiation therapy; 2 women reported success while 2 reported failure.

Summary of Data Found To Be Clinically But Not Statistically Significant and Their Implications

Table 16. Data Found to Have Clinical But Not Statistical Significance and Their Implications.

<u>Protective</u>	<u>Increased Risk of Keloid Formation</u>
AB negative Blood Group Type	HLA- B 5
HLA-A 1	HLA- B 51 (5)
HLA-A 25 (10)	HLA-B 44 (12)
HLA-A 26 (10)	HLA-B 13
HLA-Aw 66 (10)	HLA-B 14
HLA-B 8	HLA-B 62 (15)
HLA-B 16	HLA-B 18
HLA-B 17	HLA-B 21
HLA-B 57 (17)	HLA-B 49 (21)
HLA-Bw 22	HLA-B 40
HLA-B 34	HLA-Bw 60 (40)
HLA-Cw 1	HLA-Bw 48
Positive History of Cancer	HLA-Bw 6
Positive History of Diabetes	HLA-Cw 3
Penicillin Allergy	HLA-Cw 5
Sulfa Drug Allergy	Positive History of Heart Disease
Family History of Cancer	Positive History of Lung Disease
Family History of Hypertension	Positive History of Arthritis
Family History of Blood Disease	Positive History of Uterine Disease
	Subjective Report of Easy Bleeding
	Aminoglycoside Allergy
	History of Father with Keloid
	History of Sister with Keloid
	History of Any Relative with Keloid
	Family History of Epilepsy

DISCUSSION

Scarring is a phenomenon that has been of interest to patients, physicians and researchers alike for centuries. Since the scars are the only things which the patient sees, physicians have endeavored to make them as cosmetically acceptable as possible. In light of this, objectionable scarring has frustrated both the patient who must live with this for the rest of their lives, and their physicians. It is no wonder then, that considerable research interest has been paid to keloidal scarring which is not only cosmetically unappealing, but can also be disfiguring and painful.

Researchers have concentrated their efforts into studies undertaken to differentiate between keloid scars and hypertrophic scars (HTS) on the basis of their histology and biochemistry and although this has produced many differences between the two, many similarities have also been found. Often, however, in clinical practice the physician must rely on the criterion that while keloids are characterized by scar tissue which extends or overflows beyond the boundaries of the wound edges into normal tissues, HTS are raised erythematous scars which do not extend beyond the dimension of the original injury. Unfortunately, this differentiation is often not as easy to perform as it might sound and even physicians are confused as to what type of objectionable scar they are faced with in their practices. For this reason, it is often unclear as to whether the scars which have been studied in an effort to gain insight into keloidal scarring were truly keloids and not HTS or a mixture of the two.

This study was undertaken to define factors which, because of their increased frequency of occurrence in keloid patients might be thought to confer increased relative risk to the development of keloid formation. Similarly, it was hoped that factors might be found which could be considered protective against keloid formation in that they occurred with increased frequency in non-keloid formers. For this purpose, two groups of patients were defined. The keloid group consisted of Black women who had formed keloids on their earlobes as a result of earpiercing. Earlobe keloids are the only truly

indisputable form of keloids since the wound is a puncture site and any scar overflowing the boundaries of the wound edges is defined as a keloid. The control group had to be Black women free of any type of objectionable scar and had to have experienced the trauma of ear piercing to be included in this study. In this way two pure groups were formed, one with true keloids and one without any keloids. To this researcher's knowledge this is the first time a study has been performed using such stringent criteria for inclusion.

These two groups of women were first compared demographically to ensure they were matched on the basis of ethnic background and age. All of the women included in this study were Black and as can be seen by Table 1 they were of roughly the same age. An interesting and potentially confusing result from this aspect of the study was that the control group reported ages at the time of ear piercing which were significantly younger than the keloid group. If the median is used as a standard of comparison the control group was 13 years younger (12 if the mean is used) at the time of ear piercing. It has long been thought that there was an inverse relationship between age and the development of keloids (Alhady & Sivanantharajah, 1969; Ketchum et al., 1974; Ramakrishnan, 1974). It had been postulated that this tendency may be due to the fact that there is increased resiliency and increased tension in younger skin (Doyle & Passey, 1975). Aging has also been known to cause decreased inflammation, wound contraction, cellular proliferation and metabolism; decreased rates of capillary ingrowth and mast cells and decreased synthesis of collagen (Reed & Clark, 1985) and this may also have been offered as an explanation as to why keloids occurred more frequently in the young. This result then seems to go against what has been previously reported in the literature and the reasons for this are not clear.

It has also been put forth that increasing levels of estrogen may be associated with keloid formation. Support for this has been the literature which reports that keloids often develop with puberty and may have a peak incidence in the immediate post

puberty years (Moustafa & Abdel-Fattah, 1975); that there is regression of keloids with menopause (Koonin, 1964), and that keloids often appear of increase in size during pregnancy (Ramakrishnan, 1974). This may be a possible explanation of the results of this study. The median and mean ages reported by the control group (4 years and 8.54 years) are pre-pubescent years while those reported by the keloid group (17 years and 20.58) are post-pubescent years and as such this group can be expected to higher levels of circulating estrogens as well as higher levels of ongoing estrogen production. Perhaps the effects of higher levels of this hormone are greater than the tendency for keloids to form in younger groups.

Results of prior investigations of the possible association of particular ABO blood group types with keloid formation have been controversial. One study indicated that type A occurred in greater frequency in a keloid group in a population of people where type O was predominant (Ramakrishnan, 1974). The only other study reported indicated that type B was found to occur with the greatest frequency in the keloid patients studied but that type B was also the predominant blood type for the people of the area (Ghosh et al., 1979). This study sought to determine whether an ABO/Rh blood type occurred with greater frequency in the keloid or in the control group. Three of the known ABO/Rh blood types were encountered in neither group (A negative, B negative and AB negative). Appendix H contains the frequency of ABO/Rh blood group occurrence in the U.S. population. As can be seen in this table the frequency of A negative in Blacks is 4.05, that of B negative is 3, and that of AB negative is 0.6. In order to expect to get even one patient with blood type AB negative one would have to study over 200 patients. When these frequencies are considered it is not surprising that these blood groups were not encountered in either group.

An interesting result which came out of this data is that it is the blood type AB positive which was found to occur with greater frequency in the control group thus suggesting a protective effect against keloid formation. While this was not found to have

statistical significance, it is interesting in light of the fact that the frequency of its occurrence is 3.4 in U.S. Blacks and yet it was found in a sample with only 20 women in each group. This result suggests that further study using larger sample sizes may result in even statistical significance.

Neither type A nor type B were found to occur with greater frequency in keloid patients as reported previously. As was pointed out earlier the area in which type B was found to occur with greatest frequency in the keloid group was an area in which the predominant blood type was B making the result statistically insignificant.

Assessment of the ability of the control group to be considered representative of the population of Black women in terms of HLA-A and HLA-B antigen data was performed by comparison of the expected vs. observed incidences in the control group of the most commonly occurring HLA-A and HLA-B histocompatibility antigens in Blacks and this is summarized in Table 3. From this it can be seen that the control group was indeed representative of the Black population at large and could serve as a valid reference standard against which the keloid group could be compared.

The genes for HLA histocompatibility antigens are located on chromosome 6 and a table of the Class I (HLA-A, HLA-B, and HLA-C) antigens is located in Appendix B. These genes are codominantly expressed and all the antigens coded for by these genes are found on the surfaces of most of the cells of the body. These antigens have come to be known as transplantation antigens because transplants between individuals carrying different alleles at the HLA-A, HLA-B, and HLA-C loci are rapidly rejected.

In addition to their role in transplantation, susceptibility to a number of diseases has been found to correlate with an individual's HLA phenotype. It has been reported that the possession of specific HLA antigens occur in greater frequency in individuals with certain diseases in comparison to individuals without the diseases and thus the possession of these antigens is said to confer an increased relative risk of developing the disease. Perhaps the most well known of these associations is that of HLA-B 27

conferring a relative risk of 90 to the development of ankylosing spondylitis. Other, less well known, antigens which confer increased risk include:

HLA-A 2.....congenital heart malformation;
 HLA-A 3.....idiopathic hemochromatosis;
 HLA-B 5Behcet's disease;
 HLA-B 8myasthenia gravis;
 HLA-B 27.....Reiter's syndrome;
 HLA-B 27.....juvenile rheumatoid arthritis;
 HLA-Bw 35subacute thyroiditis;
 HLA-Cw 6.....psoriasis (McDevitt, 1985).

Three studies have reported possible associations of antigens of the HLA Class I Histocompatibility system with keloid development. Two of these studies conducted by Laurentaci & Dioguardi (1977; 1978) compared 40 and 25 White patients with keloids and hypertrophic scars (unfortunately they did not differentiate between these two conditions) to 131 White controls for 11 of the 24 known HLA-A antigens and 15 of the 52 known HLA-B antigens. These authors reported finding that the possession of HLA-B 14 carried a relative risk of 6.30, while the possession of HLA-Bw 16 conferred a relative risk of 3.84 (4.85 in their second study) of developing keloids. Cohen et al. (1979) studied 19 of the 24 known HLA-A antigens and 15 of the 52 known HLA-B antigens in Black keloid patients and reported that while they found the possession of HLA-A2 to confer a relative risk of 2.04 and the possession HLA-Bw 35 to be negatively correlated with keloid formation, their results were not statistically significant.

This study was much more extensive in that it investigated all of the known HLA-A and HLA-B antigens as well as the HLA-C antigens. Of these, 5 antigens were found to have results which were statistically significant and these are summarized in Table 8. HLA-A2 was found to occur in greater frequency in the control group and thus to have a protective effect on keloid formation. This is in direct contrast to work of Cohen et al. (1979) in which it was found to confer increased risk, and to the work of Laurentaci & Dioguardi (1977; 1978) in which it was found to have no relation. HLA-A 9 and one of

its narrow specificities HLA-A 23 were found to occur in increased frequency in the keloid group and thus to confer increased risk to keloid development. This is in contrast to all of the mentioned studies in which HLA-A 9 was investigated but found to have no association. HLA-Aw 34 and HLA-Cw 2 were similarly found to occur in greater frequency in the keloid group and thus confer increased risk. The Cohen study- (1979) found no difference in the frequency of occurrence of Aw 34 in its keloid and control group; and neither study examined HLA-Cw antigens. Interestingly, no HLA-B antigen was found to have statistically significant results.

Several HLA-A, HLA-B, and HLA-C antigens occurred in frequencies which clinically suggested either protective effects on the development of keloids or increased risk of their development but did not achieve statistical significance. These are summarized in Table 16. Four HLA-A antigens were found to be protective including A1, A 25 (10), A 26 (10), Aw 66 (10). All of these, except for Aw 66 which had not been previously studied, had in prior investigations been found to have no association with keloid development. Eleven HLA-B antigens were found to be clinically protective including, interestingly, HLA-B 16 which had been reported to confer increased risk. HLA-Cw1 was similarly suggestive of protective effects against keloid formation. Thirteen B antigens were found in frequencies which suggested increased risk including HLA-B 14 reported previously to increase risk. Two HLA-Cw antigens were similarly found in frequencies which conferred increased risk.

Several factors may be responsible for the differences found between this study and previous investigations. In the two studies which did result in statistically significant findings the population of patients which were studied consisted of White keloid and HTS patients. The frequencies of occurrence of HLA histocompatibility antigens differ among people in different ethnic backgrounds. This could account for why the Laurentaci & Dioguardi study did not find A2 to be a significant factor. It must also

be considered that the first two studies did not have a pure keloid group and that the purity of the keloid patients in the Cohen study is uncertain.

There are a relatively large number of HLA histocompatibility antigens which achieved clinical but not significant significance. It must be remembered that over 75 antigens were analyzed in two groups consisting of 20 patients each. These clinically significant results should be considered to be suggestive of association between the antigens and keloid formation. These might serve as a basis for future investigation.

Previous literature had suggested that certain medical conditions exhibited effects on the process of wound healing. Diabetes has been reported to result in wound hypoxia and microangiopathy and major arterial occlusion secondary to accelerated arteriosclerosis (Carrico et al., 1984). However, the effects of this on keloid development have never been elucidated. It was once felt that tuberculosis and syphilis were associated with keloid formation but this is no longer felt to be true (Koonin, 1964). Other possible associations between keloid development and medical conditions had not been explored.

In this study the single medical condition which was found to be both clinically significant and statistically significant was a report of a positive history of hypertension. This was reported in greater frequency in the keloid group suggesting an increased risk of development of keloids in patients with hypertension. Unfortunately, why these should be associated is not clear. Hypertension is a disease with many causes and many associated risk factors. This result is also difficult to interpret based on the fact that it is uncertain whether the patient had developed hypertension prior to earlobe piercing or not.

Several conditions were found to occur with greater frequency in the keloid group, thus suggesting an increased risk. These conditions included a positive history of heart disease, uterine disease, blood disease, lung disease, arthritis and a subjective sense of easy bleeding (not worked up through bleeding studies). These results,

however, did not reach statistical significance. Similarly it is difficult to interpret why these medical conditions should exhibit protective effects. The associations of these may be the basis of future investigation.

Interestingly, although diabetes has been reported to adversely affect wound healing, its association with keloid formation has never been made clear. In this study a positive history of diabetes was found to occur in greater frequency in the control group suggesting a protective effect. It might be hypothesized that the fact that diabetes results in wound hypoxia which deprives the wound of oxygen necessary for collagen synthesis (Reed & Clark, 1985; Carrico et al., 1984) may be at least partially responsible for this protective association. It could also be suggested that the fact that the resultant hypoxia decreases cell migration and proliferation (Reed & Clark, 1985; Carrico et al., 1984) may be a partial explanation. Although this result did not reach statistical significance in light of the large number of patients affected by diabetes and keloids the association between these two entities deserves further investigation.

A finding which is quite puzzling is the increased frequency of occurrence of a positive history of cancer in the control group suggesting a protective effect. It would be interesting to see if this relationship holds in a sample of larger size.

Histamine has been found to exhibit several effects on wound healing. It has been suggested that histamine may be a stimulant for collagen formation (Cohen et al., 1972) and it has been demonstrated that it can stimulate cell growth in the human fibroblast (Topel et al., 1981). HTS and keloids have also been shown to have increased levels of histamine which parallel the rate of collagen synthesis in these scars (Cohen et al., 1972). It has also been demonstrated that histamine increases the permeability of capillaries and thus modulates the hemostatic and inflammatory phases of scar production (Boucek, 1984). In addition to these things histamine is intimately associated with allergies, being the substance which is released from the mast cell during the allergic reactions.

This is an interesting forum in which to examine the results obtained from questioning the groups on their history of allergies. A positive history of allergies to substances other than drugs was found to occur in greater frequencies in the control groups suggesting a protective effect which was both clinically and statistically significant. Similarly positive histories of allergies to penicillin and sulfa drugs were found to have clinically protective significance although these results did not reach statistical significance. A positive history of allergy to aminoglycosides was found to occur in greater frequency in keloid patients indicating an increased risk which was not statistically significant. Sample size is too small to reconcile this data in light of previous literature.

Local wound infection has long been known to prolong the inflammatory phase and hence prolong wound healing (Reed & Clark, 1985). It is still unclear as to whether the effects of local infection on wound healing are a result of the presence of bacteria in the wound or a host reaction to their presence. In light of this patients were asked to report the occurrence of local infection at the site of ear piercing to examine the association between wound infection and keloid formation. A positive history of infection after ear piercing occurred in greater frequency in the keloid group suggesting an increased risk in keloid formation. This would seem to indicate that the prolongation of the inflammatory phase can result in keloid formation.

It has long been suggested that there may be genetic predisposition for keloid formation. Ramakrishnan (1974) reported 19 families with multiple cases of keloids among the 1000 cases of keloids that he studied. Koonin (1964) suggested that there may be a hereditary physiochemical difference in keloid prone families which cause the overproduction of connective tissue at sites of injury called fibroplastic diathesis. Patients in both groups of this study were asked to report a positive family history of keloid formation in any of their relatives and to name the relative. The results indicated that a positive report of keloid formation in any family member occurred in greater

frequency in the keloid group and furthermore that the family members reported to have keloids were either a father or sister. These results were not statistically significant but suggested an increased risk of keloid development if any relative, a father or a sister had a positive history of keloid formation. These results would appear to support the existing literature on the genetic predisposition of keloids. The inheritance pattern is far from determined but it might be suggested that keloid formation may be an X-linked recessive trait. This would explain an increased frequency of occurrence in a father or sister, but not a mother or brother.

Associations between keloid formation and family history of medical conditions have never been investigated. The results of this study indicated that a positive family history of tuberculosis was reported in greater frequency in the control group suggesting a protective effect which was found to be statistically significant. A positive family history of cancer, hypertension and blood diseases were also reported with greater frequency in the control group indicating a protective effect but these results were not found to be statistically significant. It is interesting that while a positive family history of hypertension was found to be protective a personal history of hypertension was found to confer increased risk. A positive family history of epilepsy occurred more frequently in the keloid group indicating increased risk, however, this was not found to be statistically significant.

Studies have never been conducted on the progression of keloid formation among women. When asked to recall any incident involving their earlobes between the time of ear piercing and the initial onset of keloid formation the majority of keloid formers reported no incident, while the remaining reported either infection or injury to the ear in the form of trauma secondary to pulling of earrings or bites to the ear. When asked to recall the interval of time between ear piercing and the onset of keloid formation the majority of women reported an interval of greater than one year, followed by reports of less than one month. This would seem to indicate that there are two time frames during

which women are most susceptible to the development of keloids, less than one month after injury or greater than one year. What events occur during these periods of time which make them more susceptible are still in question.

Although the goal of this study was to investigate potential risk factors and protective factors in keloid formation the opportunity was taken to investigate the locations of keloids in women with multiple keloids and to assess therapeutic efficacy. Areas of high risk for keloid formation have been reported in the literature and include the presternum, upper back, shoulders, neck face, earlobes and anterior chest wall (Koonin, 1964; Crockett, 1964; Alhady & Sivanantharajah, 1969). Low risk areas include the eyelids, genitalia, palms, soles, lower back and lower limb (Crockett, 1969). The data from this study indicate that the keloid formers reported the majority of their other keloids occurring on the their abdomens, followed closely by their upper extremities. The earlier literature did not discuss the abdomen as a primary site of keloid formation but did indicate the upper extremity as a primary site. In keeping with the literature the lower limb and lower back were reported with much less frequency and appeared to corresponding be low risk areas.

Finally the efficacy of past therapeutic interventions were assessed. The patients reported that all women who attempted surgical excision and local steroid injection experienced a successful outcome in keeping with the literature which reported this combination as being one of high therapeutic efficacy. On the other hand the majority of women who attempted surgical excision alone experienced recurrence of their keloids in keeping with the literature that reported a low rate of success for this therapeutic intervention.

This thesis was undertaken to investigate possible predictive factors in keloid formation and determine any protective factors which may exist. The results of this work indicates that there are several factors which occur in greater frequency in Black

women with keloids that have been statistically found to confer increased risk for keloid formation. These factors include:

- (1) an older age at the time of earpiercing
- (2) possessing the HLA-A9 antigen
- (3) possessing the HLA-A 23 (9) antigen
- (4) possessing the HLA-Aw 34 antigen
- (5) possessing the HLA-Cw 2 antigen
- (6) a positive history of hypertension

Factors have also been found have occurred in increased frequency in the control group indicating a protective effect which achieved statistical significance. These factors include:

- (1) possession of HLA-A 2 antigen
- (2) a positive history of allergies to substances other than drugs
- (3) a positive family history of tuberculosis

Many other factors were found to reach clinical significance in this study but not statistical significance. However, it should be kept in mind that the number of subjects in the study were small and for that reason these factors should not be dismissed as insignificant. Factors have been found that clinically indicate protective effects against keloid formation as well as factors which have been found to clinically indicate increased risk. These factors are summarized in Table 16.

It should be realized that this investigation is only a start in determining factors which may be used to predict future keloid development. It is the hope of this investigator that these results be used as a base for areas of future investigation. With continued research it may one day be possible to predict which patients are susceptible to keloid development prior to elective surgical procedures and take post-operative steps including local steroid injection to minimize disfiguring and painful keloidal scarring.

APPENDIX A
INFORMATION FOR CASE HISTORY FILE
(PLEASE complete **all items**. PLEASE print.)

Date _____

Subject's Name _____ Age _____ Date of Birth _____

Home Address _____ City _____ State _____ Zip _____

Home Phone _____ Work Phone _____

Parent's Name _____ Work Phone _____

Subject Status: KELOID FORMER NON-KELOID FORMER

KELOID HISTORY

Dimensions: _____

Age at ear piercing? _____

What kind of keeper was placed in ear? None ____ Stainless Steel ____ Gold ____
Silver ____ Other _____

Did you take any medications immediately after having your ear pierced?

Yes ____ No ____

If yes please list: Aspirin ____ Tylenol ____ Steroids ____ Others _____

What was the reaction of ear after piercing? No reaction ____ Redness ____

Swelling ____ Discharge ____ Other _____

Did you experience infection in ear after piercing? Yes ____ No ____

Was scarring associated with any incident other than the ear piercing alone?

No ____ Yes ____

If Yes: Injury to ear ____ Later infection ____ Other _____

How long after piercing did you notice the beginning of scarring? 0-1 month ____

1-3 months ____ 3-6 months ____ 6-9 months ____ 9mon-1 year ____ >1 year ____

Do you have any keloids other than the earlobe keloid? Yes ____ No ____

If yes please list all other keloids and the incidents surrounding their occurrence:

(example : Result of Surgery, vaccination, trauma or injury)

#1 Area of Body _____ Result of _____

#2 Area of Body _____ Result of _____

#3 Area of Body _____ Result of _____

#4 Area of Body _____ Result of _____
 #5 Area of Body _____ Result of _____
 #6 Area of Body _____ Result of _____
 #7 Area of Body _____ Result of _____

Were you taking any medications at the time of keloid formation? Yes ___ No ___

If yes, please list: Aspirin ___ Tylenol ___ Steroids ___ Others _____

Please list ALL the treatments have you sought for your earlobe or other keloids? Please be specific and give the dates on which treatments were begun and ended. For this question success means that keloid did not recur and failure means that keloid did recur.

Surgery alone: No ___ Yes ___ Date _____ Succeeded _____ Failed _____

Steroids alone: No ___ Yes ___ Date _____ Succeeded _____ Failed _____

Surgery and Steroids: No ___ Yes ___ Date _____ Succeeded _____ Failed _____

Radiation alone: No ___ Yes ___ Date _____ Succeeded _____ Failed _____

Surgery and Radiation: No ___ Yes ___ Date _____ Succeeded _____ Failed _____

Other: No ___ Yes ___ Date _____ Succeeded _____ Failed _____

Explain the procedure attempted _____

Have you ever consulted any doctors other than your present one about your keloids? If yes please list their names and affiliations.

HAIR CARE HISTORY

Please describe the condition you feel your hair is in: Dry ___ Normal ___ Oily ___
 Healthy ___ Damaged ___

Do you have trouble with hair breakage? Yes ___ No ___

Do you dye or streak your hair? Yes ___ No ___ If yes when was the last time you dyed your hair? _____

Do you chemically process your hair in any way? (Do you use relaxers, curl activators, etc.?) Yes ___ No ___

If so please list what you or your hair stylist uses on your hair.

 When was the last time the chemicals described above were put on your hair?

 Do you have dandruff? Yes ___ No ___

What brand of shampoo and conditioner do you use? _____

What other hair products do you use? Please list all.

MENSTRUAL AND CONTRACEPTIVE HISTORY

What was the date of your last menstrual period? _____

Would you describe your menstrual cycle as regular? Yes ____ No ____ If no please explain:

What type of contraceptives do you use? Please list all.

PAST MEDICAL HISTORY

General Health: Good ____ Fair ____ Poor ____

If not "Good", please explain

Height _____ Weight _____ Weight loss or gain in past year _____ lbs.

How long ago was your most recent physical check-up?

Please list any past serious illnesses:

Tuberculosis.....	No__	Yes__
Cancer.....	No__	Yes__
Diabetes.....	No__	Yes__
Epilepsy.....	No__	Yes__
Heart Disease.....	No__	Yes__
High Blood Pressure.....	No__	Yes__
Blood or Bleeding Disorders.....	No__	Yes__
Asthma.....	No__	Yes__
Lung Disease.....	No__	Yes__
Kidney Disease.....	No__	Yes__
Gastrointestinal Disease.....	No__	Yes__
Arthritis.....	No__	Yes__
Scarlet Fever.....	No__	Yes__
Rheumatic Fever.....	No__	Yes__
Skin Diseases (hives, eczema, rash).....	No__	Yes__
None.....	Yes__	

Please list any other chronic or recurring infections or illness.

PREVIOUS SURGERY (PLEASE LIST)

[illegible]

Have you had significant complications or after effects from any of these operations?

Yes ____ No ____ If yes please

explain. _____

PAST INJURIES

Type	Year	Did you form a keloid later?
------	------	------------------------------

[illegible]

FAMILY HISTORY

	Age	State of Health	Have they ever formed keloids?
Mother	_____	_____	_____
Father	_____	_____	_____
Brother(s)	_____	_____	_____

Sister(s) _____

Children

Has any relative ever had:

Tuberculosis.....	No___	Yes___
Cancer.....	No___	Yes___
Diabetes.....	No___	Yes___
Epilepsy.....	No___	Yes___
Heart Disease.....	No___	Yes___
High Blood Pressure.....	No___	Yes___
Blood or Bleeding Disorders.....	No___	Yes___
Asthma.....	No___	Yes___
Lung Disease.....	No___	Yes___
Kidney Disease.....	No___	Yes___

MEDICATIONS, DRUGS

What is your approximate daily consumption of the following:

Coffee or Tea _____

Tobacco _____

Alcohol _____

Other intoxicating or mind altering drugs. Please specify.

Please list all medications you are now taking:

Birth control pills Yes ___ No ___ Hormones Yes ___ No ___

Diuretics (water pills) Yes ___ No ___ Tranquilizers Yes ___ No ___

Blood pressure medication Yes ___ No ___ Aspirin Yes ___ No ___

Tylenol Yes ___ No ___ Steroids Yes ___ No ___

Heart medication Yes ___ No ___ Others Yes ___ No ___ List: _____

Did you take any of these immediately after having your ears pierced? Yes ___ No ___

If Yes, please list which. _____

Please list any of above drugs taken during treatments for your keloids _____

OTHER PERTINENT INFORMATION

Are you allergic to any medicines? No ___ Yes ___

If yes, which ones?

Are you allergic to anything besides medicines? No ___ Yes ___ If yes, what?

Do you bleed unusually easily (from cuts, surgery, tooth extractions)?

Yes ___ No ___

Do you bruise unusually easily? Yes ___ No ___

Are you a slow or poor healer? Yes ___ No ___

Do you have frequent infections or boils? Yes ___ No ___

Have you taken steroid medications, cortisone, or ACTH? Yes ___ No ___

Do you have shortness of breath with walking? Yes ___ No ___

Have you had any illness or disorders of the following? (Circle if Yes)

Brain Face Lungs Intestines Blood

Bones/Joints Eyes Nose, Sinus, Throat Liver

Reproductive System Arms/Legs Ears Breasts Stomach Urinary System

Nervous System Endocrine/Diabetes

If circled, please explain.

Signature _____

Relationship to Subject _____

APPENDIX B

HLA CLASS I (A, B, C) NOMENCLATURE

A 1	B 5	B 49 (21)	Cw 1
A 2	B 7	Bw 50 (21)	Cw 2
A 3	B 5	B 51 (5)	Cw 3
A 9	B 12	Bw 52 (5)	Cw 4
A 10	B 13	Bw 53	Cw 5
A11	B 14	Bw 54(w22)	Cw 6
Aw 19	B 15	Bw 55 (w22)	Cw 7
A 23 (9)	B 16	Bw 56 (w22)	Cw 8
A 24 (9)	B 17	Bw 57 (17)	Cw 9 (w3)
A 25 (10)	B 18	Bw 58 (17)	Cw 10 (w3)
A 26 (10)	B 21	Bw 59	Cw 11
A 28	Bw 22	Bw 60 (40)	
A 29 (w19)	B 27	Bw 61 (40)	
A 30 (w19)	B 35	Bw 62 (15)	
A 31 (w19)	B 37	Bw 63 (15)	
A 32 (w19)	B 38 (16)	Bw 64 (14)	
Aw 33 (w 19)	B 39 (16)	Bw 65 (14)	
Aw 34 (10)	B 40	Bw 67	
Aw 36	Bw41	Bw 70	
Aw 43	Bw 42	Bw 71 (w70)	
Aw 66 (10)	B 44 (12)	Bw 72 (w70)	
Aw 68 (28)	B 45 (12)	Bw 73	
Aw 69 (28)	Bw 46	Bw 75(15)	
Aw 74 (w19)	Bw 47	Bw 76(15)	
	Bw 48	Bw 77 (15)	
		Bw 4	
		Bw 6	

* Antigens in parentheses () denote "parent" antigens (broad specificities) from which the new specificities (narrow specificities) have "split".

Recently adopted nomenclature of the Tenth Annual Workshop, New York, 1987

TERASKAI SECOND HLA-ABC TRAY ANTIGEN SPECIFICITY CODING SHEET

TERASAKI SECOND ILLA (72) WELL TRAY, LOT #5

(CATALOG #T2-72) 4/88

COMPLEMENT LOT____ EXP____

4/08

LAST NAME

FIRST

CENTER

INVESTIGATOR

_____ BLEEDING DATE

SEX

RACE

ABO

DISEASE

ANTIC

ANTIGEN GROUPS OBTAINED

REMARKS

[illegible]

ROW	7						8						9						10						11						12					
COL NO	A	B	C	D	E	F	F	E	D	C	B	A	A	B	C	D	E	F	F	E	D	C	B	A	A	B	C	D	E	F	F	E	D	C	B	A
REACTION																																				
GROUP	44	45	13	13	14	14	75 15 57	15 75	62	16	39	63	17	17	18	18	21	49	22	42	54 53	56	27 47	27 53	35 53	35 53	37 47	40 48	40 48	60 61	41 60	41 60	41 60	41 60	41 60	41 60
SERUM	M3318	M3747	M0137.B0	M3142.I0	M3746.A0	M3075	M6035.A0	M3527.00	M2952.B0	M5042.B0	M3319	M2141	M0082.B0	M4968	M3509	M5315.A0	M5387	M5400.F0	M5037.D0	M4946.B0	M2175.B0	M2944.B0	M5451	M4044	M5096	M5199	M1696	M5746	M5450	M55749	M2573	M3301.B0	M2872	M4447	M2569	

APPENDIX D

TERASAKI BLACK HLA-ABC TRAY ANTIGEN SPECIFICITY CODING SHEET

TERASAKI HLA-ABC SUP-BLACK TRAY, LOT #5

(CATALOG #BL -60) 4/88

COMPLEMENT LOT#

EXP

(Supplemental Tray)

LAST NAME _____ FIRST _____ CENTER _____ INVESTIGATOR _____

ROW	1						2						3						4						5						REMARKS
	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F	
COL:ND																															
REACTION																															
GROUP																															
SERUM																															

ROW	6						7						8						9						10						REMARKS
	F	E	D	C	B	A	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F	F	E	D	C	B	A	
COL:ND																															
REACTION																															
GROUP																															
SERUM																															

APPENDIX E

Coding Sheet

Col. #	Variable	Code	
	Pt. ID # (1-100)	--	--
B-K 1	Keloid Status [Control (0) Keloid (1)]	--	--
B-K2	Pt. Age		--
B-K3	ABO-Rh type		--
	A+ (1) B+ (2) O+ (3) AB+ (4)		
	A- (5) B- (6) O- (7) AB- (8)		--
	HLA type		
B-K4	A		--
B-K5	A		--
B-K6	B		--
B-K7	B		--
B-K8	Bw		--
B-K9	Bw		--
B-K10	Cw		--
B-K11	Cw		--
B-K12	Age at ear piercing		--
B-K13	Keeper placed in ear		
	none (0) Stainless steel (1) Gold (2)		
	Silver (3) Other (4)		--
B-K14	Meds after piercing		
	none (0) aspirin (1) tylenol (2) steroids (3)		
	other (4)		--
B-K15	Reaction after piercing		
	no reaction (0) redness (1) swelling (2)		
	discharge (3) all (4) 1&2 (5) 1&3 (6)		
	2&3 (7) other (8)		--
B-K16	Infection post ear piercing [no (0) yes (1)]		--
B-K17	Scarring assoc with: nothing (0) injury (1) infection (2)		
	other (3)		--
B-K18	Time post piercing scarring begun		
	<1 mo (1) 1-3 mo (2) 3-6 mo (3) 6-9 mo (4)		
	9mo-1yr (5) >1 yr (6)		--
B-K19	Number of body keloids other than earlobe		--
	Location of body keloids(# in area)		
B-K20	head		--
B-K21	neck		--
B-K22	chest		--
B-K23	abdomen		--
B-K24	upper extremity		--
B-K25	lower extremity		--
B-K26	other		--
	Incidence surrounding keloid occurrence(#due to)		
B-K27	surgery		--
B-K28	ear piercing		--

B-K29	burn	---
B-K30	laceration not req. stitching (LAC-STIT)	---
B-K31	laceration req. stitching (LAC + STIT)	---
B-K32	pimple	---
B-K33	other	---
T-D-K2	Meds at time of keloid formation none (0) aspirin (1) tylenol (2) steroids (3) other (4)	---
	Keloid therapies tried and success data	---
	successful surgery only (1)	---
	successful steroid only (2)	---
T-D-K3	successful surgery + steroid (3)	---
	successful radiation only(4)	---
	successful radiation and surgery (5)	---
	successful other (6)	---
T-D-K4	unsuccessful surgery only no (0) yes (1)	---
T-D-K5	unsuccessful steroid only no (0) yes (1)	---
T-D-K6	unsuccessful surgery + steroid no (0) yes (1)	---
T-D-K7	unsuccessful radiation only no (0) yes (1)	---
T-D-K8	unsuccessful radiation and surgery no (0) yes (1)	---
T-D-K9	unsuccessful other no (0) yes (1)	---
	Past serious illnesses	
T-D-K10	TB no(0) Yes (1)	---
T-D-K11	Ca no(0) Yes (1)	---
T-D-K12	diabetes no(0) yes (1)	---
T-D-K13	epilepsy no (0) yes (1)	---
T-D-K14	heart Dz no(0) yes (1)	---
T-D-K15	blood or bleeding dz no (0) yes (1)	---
T-D-K16	asthma no(0) yes (1)	---
T-D-K17	lung dz no(0) yes (1)	---
T-D-K18	kidney dz no(0) yes (1)	---
T-D-K19	GI dz no (0) yes (1)	---
T-D-K20	arthritis no (0) yes (1)	---
T-D-K21	scarlet fever no(0) yes (1)	---
T-D-K22	rheumatic fever no (0) yes (1)	---
T-D-K23	skin dz (hives, eczema, rash) no(0) yes (1)	---
T-D-K24	none yes (0) no (1)	---
T-D-K25	Chronic Illnesses or Infections no(0) yes (1)	---
T-D-K26	Number of operations w/ Keloid formation	---
T-D-K27	Number of operations w/o Keloid formation	---
T-D-K28	Number of serious past injuries w keloid formation	---
T-D-K29	Number of serious past injuries w/o keloid formation	---
T-D-K30	HTN no (0) yes (1)	---
F-M2	Number of first degree relatives who have formed keloids	---
	Family members who formed keloids:	
F-M3	mother no (0) yes (1)	---
F-M4	father no (0) yes (1)	---
F-M5	brother no(0) yes (1)	---
F-M6	sister no(0) yes (1)	---
F-M7	children no (0) yes (1)	---

F-M8	other no (0) yes (1)	--
	Family history of:	
F-M9	TB no (0) Yes (1)	--
F-M10	Ca no (0) yes (1)	--
F-M11	Diabetes no (0) yes (1)	--
F-M12	Epilepsy No (0) yes (1)	--
F-M13	Heart Dz no (0) yes (1)	--
F-M14	HTN no (0) yes (1)	--
F-M15	Blood or bleeding Dz no (0) yes (1)	--
F-M16	Asthma no (0) yes (1)	--
F-M17	Lung Dz no (0) yes (1)	--
F-M18	Kidney Dz no (0) yes (1)	--
F-M19	Tobacco Consumption	
	[non-smoker (0) smoker (1)]	--
F-M20	Caffeine Consumption	
	None (0) 1-3 cups per day (1)	
	4-6 cups per day (2) 7> cups per day (3)	--
F-M21	Alcohol consumption	
	none (0) social drinker (1) one drink/day (1)	
	2-4 drinks/day (2) 3> drinks/day (3)	--
F-M22	Recreational Drugs no (0) yes (1)	--
	Medications	
F-M23	birth control pills no (0) yes (1)	--
F-M24	diuretics no (0) yes (1)	--
F-M25	blood pressure no (0) yes (1)	--
F-M26	tylenol no (0) yes (1)	--
F-M27	heart no (0) yes (1)	--
F-M28	hormones no (0) yes (1)	--
F-M29	tranquilizers no (0) yes (1)	--
F-M30	aspirin no (0) yes (1)	--
F-M31	steroids no (0) yes (1)	--
F-M32	others no (0) yes (1)	--
A-D2	Allergies - Meds	
	penicillin (1) streptomycin (2) Sulfa drugs (3)	
	other (4)	--
A-D3	Other Allergies	
	hay fever (1) food (2) grass and dust (3)	
	animal (4) other (5)	--
A-D4	Bleed easily [no (0) yes (1)]	--
A-D5	Bruise easily [no (0) yes (1)]	--
A-D6	Slow/poor healer [no (0) yes (1)]	--
A-D7	Frequent infections or boils [no (0) yes (1)]	--
A-D8	Shortness of breath while walking [no (0) yes (1)]	--
	Have had disorders of	
A-D9	none yes (0) no (1)	--
A-D10	brain no (0) yes (1)	--
A-D11	face no (0) yes (1)	--
A-D12	lungs no (0) yes (1))	--
A-D13	intestines no (0) yes (1)	--
A-D14	blood no (0) yes (1)	--
A-D15	bones/joints no (0) yes (1)	--
A-D16	eyes no (0) yes (1)	--
A-D17	nose, sinus, throat no (0) yes (1)	--
A-D18	liver no (0) yes (1)	--

A-D19	reproductive system no (0) yes (1)	--
A-D20	arms/legs no (0) yes (1)	--
A-D21	ears no (0) yes (1)	--
A-D22	breasts no (0) yes (1)	--
A-D23	stomach no (0) yes (1)	--
A-D24	urinary system no (0) yes (1)	--
A-D25	nervous system no (0) yes (1)	--
A-D26	endocrine diabetes no (0) yes (1)	--

APPENDIX F

EXPECTED ANTIGEN INCIDENCE DERIVED FROM POPULATION ANALYSIS OF MAJOR HLA-A
AND HLA-B ANTIGENS

The following table displays results of population analysis for selected HLA-A and HLA-B antigens. Broad specificities (in parentheses) are listed after narrow specificities, e.g., HLA-Aw23(9) and HLA-Aw24(9).

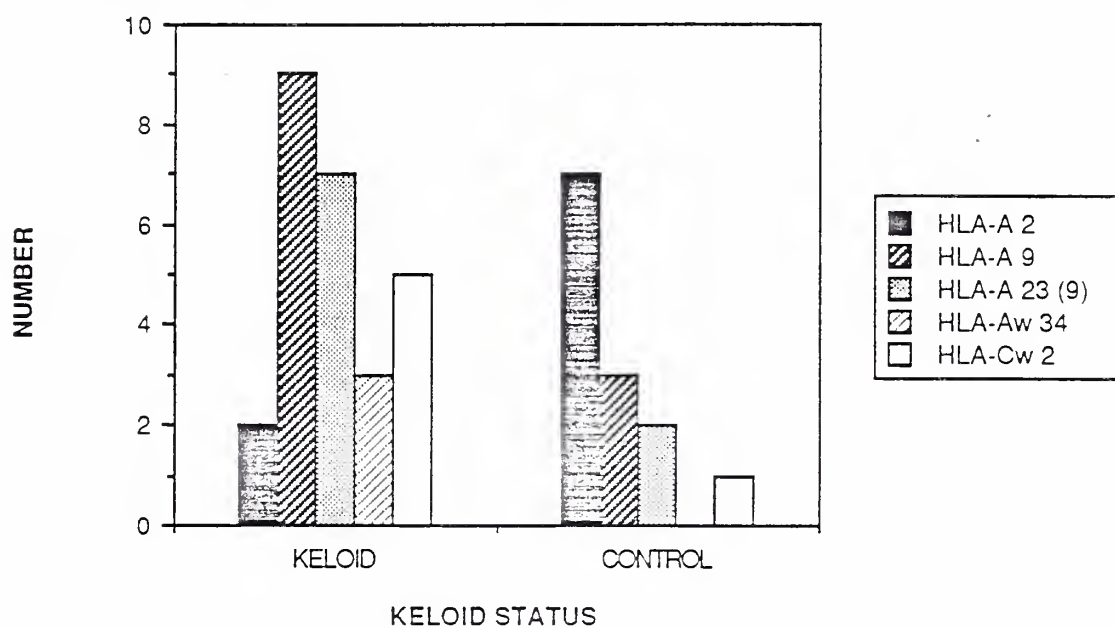
Antigen	EUC	NAC	AAC	NEG	JAP	AMI	MEX	ASH
A1	27.5	25.7	33.7	6.5	1.0	2.9	18.8	20.9
A2	45.3	46.6	41.9	27.3	43.2	60.3	44.3	40.3
A3	21.9	26.0	24.4	14.2	1.1	2.9	5.9	16.3
A11	11.5	12.5	12.9	1.1	17.2	1.5	7.1	10.9
Aw23(9)	4.5	5.0	4.7	20.4	1.1	1.5	1.2	7.0
Aw24(9)	18.2	12.8	14.0	5.7	53.5	41.9	25.9	19.4
A25(10)	3.7	4.2	7.0	0.8	0.1	0.0	21.2	1.6
A26(10)	7.2	7.2	5.8	7.4	18.7	0.0	11.8	21.7
A28	7.7	9.9	9.9	16.6	1.1	13.0	5.9	7.0
A29	7.4	8.1	8.7	12.3	0.4	4.4	10.6	7.8
Aw30	4.7	5.1	1.7	28.3	0.3	0.0	3.5	5.4
Aw31	5.4	6.2	5.8	4.4	15.3	39.0	12.9	4.7
Aw32	8.8	7.1	8.1	3.0	0.1	4.4	8.2	4.7
Aw33	3.3	3.4	0.0	9.0	13.1	4.4	8.2	6.2
Aw34	1.2	0.5	1.2	12.5	1.9	0.0	2.4	3.9
Aw36	0.7	0.7	1.2	3.3	0.5	0.0	0.0	0.0
Aw43	0.0	0.2	0.0	1.9	0.0	0.0	0.0	0.0
B7	16.8	18.7	20.9	17.0	11.4	1.5	6.0	7.8
B8	15.7	17.1	23.8	5.8	0.2	2.9	6.0	8.5
B13	5.6	5.3	4.7	1.4	4.0	0.0	4.8	5.4
B14	5.8	9.5	11.5	8.0	0.2	1.5	4.8	23.3
B18	11.2	9.7	7.0	7.7	0.0	2.9	0.0	4.7
B27	7.7	7.5	10.0	3.0	0.8	1.5	4.8	1.6
Bw35	18.2	15.6	12.2	12.1	14.0	20.3	41.7	7.9
B37	3.0	3.2	2.9	0.3	1.1	0.0	1.2	4.7
Bw38(w15)	5.0	6.2	2.9	0.0	0.4	1.5	8.3	24.8
Bw39(w16)	4.1	3.6	1.7	3.6	5.7	21.7	7.1	3.9
Bw41	2.0	3.9	1.7	2.5	0.7	0.0	1.2	8.5
Bw42	0.6	0.6	0.5	14.3	1.2	0.0	0.0	0.0
Bw44(12)	20.7	25.4	23.3	13.7	12.5	5.8	34.5	9.3
Bw45(12)	2.2	1.4	1.7	7.7	0.3	1.5	0.0	0.8
Bw47	0.9	0.4	1.7	0.3	0.4	0.0	0.0	0.6
Bw48	1.0	1.3	0.5	2.2	4.6	4.4	7.1	3.1
Bw49(w21)	4.5	4.7	2.9	4.9	0.5	1.5	1.2	0.8
Bw50(w21)	2.5	2.6	0.0	1.4	0.0	0.0	14.3	3.9
Bw51(15)	13.9	9.3	6.4	2.7	15.9	43.5	13.1	6.2
Bw52(15)	2.9	2.8	1.7	1.9	20.5	4.4	3.6	10.1
Bw53	1.7	0.9	2.9	12.6	0.2	1.5	0.0	0.0
Bw54(w22)	0.0	0.0	0.0	0.0	14.1	0.0	0.0	0.8
Bw55(w22)	4.4	4.3	5.2	1.6	5.3	0.0	0.0	4.7
Bw56(w22)	1.1	1.1	1.7	0.0	2.2	0.0	0.0	0.8
Bw57(17)	6.2	7.2	8.1	7.7	0.0	0.0	1.2	7.0
Bw58(17)	2.2	2.2	1.2	20.3	1.7	0.0	2.4	6.2
Bw59	0.9	0.8	1.2	1.6	4.2	0.0	0.0	0.8
Bw60(40)	6.7	11.0	14.5	2.7	12.7	18.8	7.1	2.3
Bw61(40)	3.3	2.0	2.9	0.8	16.3	10.1	3.5	1.6
Bw62(15)	10.4	9.5	12.2	1.9	16.7	40.5	10.7	1.6
Bw63(15)	1.0	1.9	0.0	0.6	0.4	2.9	7.1	3.1

EUC = European Caucasian, NAC = North American Caucasian, AAC = Australian Caucasian, NEG = Negro, JAP = Japanese, AMI = American Indian, MEX = Mexican, ASH = Jewish-Ashkenazi.

Antigen frequencies from Histocompatibility Testing 1980, Los Angeles: UCLA Tissue Typing Laboratory, 1980, p. 962.

APPENDIX G

Number of Patients with Statistically Significant HLA Antigens



APPENDIX H

FREQUENCY OF ABO/Rh BLOOD GROUP OCCURRENCE IN THE U.S. POPULATION

<u>ABO/Rh Group</u>	<u>Frequency (%) in U.S. Population</u>			
	<u>Blacks</u>	<u>Whites</u>	<u>Am. Indians</u>	<u>Orientals</u>
O POSITIVE	41.65	38.25	67.15	34.0
O NEGATIVE	7.35	6.75	11.85	6.00
A POSITIVE	22.95	34.0	13.6	23.8
A NEGATIVE	4.05	6.00	2.4	4.2
B POSITIVE	17.0	9.35	3.4	22.95
B NEGATIVE	3.0	1.65	0.6	4.05
AB POSITIVE	3.4	3.4	<0.85	4.25
AB NEGATIVE	0.6	0.6	<0.15	0.75

* Adopted from the Technical Manual of the American Association of Blood Banks: Ninth Edition, 1985. Frances K. Widmann Editor. Arlington, VA. pp. 114 and 127.

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